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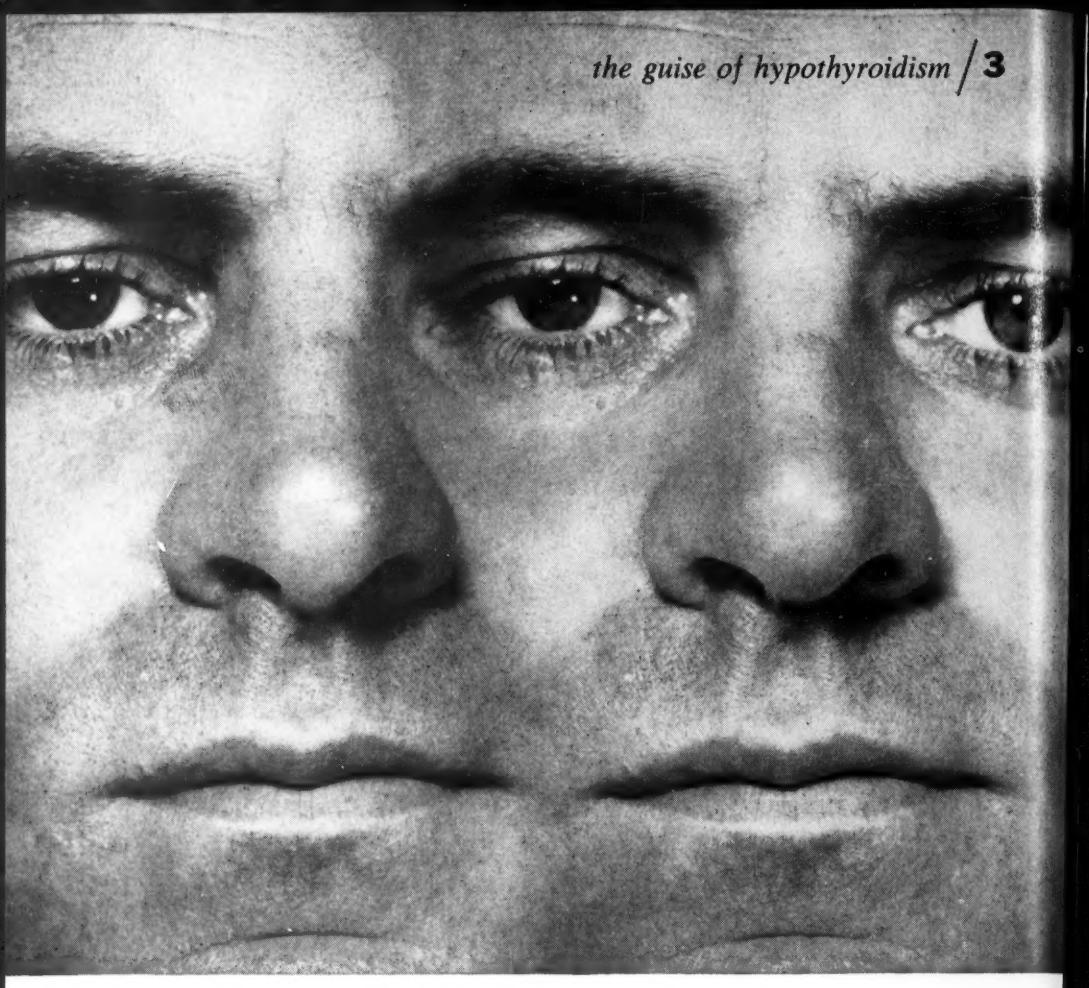

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1. Starr, P.: Postgrad. Med. *17*:73 (Jan.) 1955. 2. Pickering, D. E., and Lusted, L. B.: GP *11*:99 (Feb.) 1955.

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AN EVALUATION OF VARIOUS FACTORS INFLUENCING THE TREATMENT OF METASTATIC THYROID CARCINOMA WITH I^{131}*†

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ALTHOUGH radioactive iodine has been effective in the treatment of patients with hyperthyroidism, its usefulness has been limited in patients with metastatic thyroid carcinoma. An explanation for the latter is the failure of most thyroid carcinomas to concentrate sufficient I^{131} to be of therapeutic value. Furthermore, even in the tumors which do concentrate significant amounts of I^{131}, the response has been disappointing. Since 1945, various measures have been employed to induce or increase the uptake of I^{131} in metastatic thyroid carcinomas of patients at the Massachusetts General Hospital (MGH) prior to radioactive iodine treatment (1, 2, 3). In some of these patients large doses of I^{131} have been

* Presented at the Annual Meeting of the American Goiter Association, Oklahoma City, Okla., April 28–30, 1955.

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† Supported by the HNC gift of Harvard University, a grant from Parke-Davis & Co. of Detroit, Michigan, grants from the Atomic Energy Commission and the American Cancer Society, and by an institutional grant to the Massachusetts General Hospital from the American Cancer Society.

TABLE 1. SURGICAL THYROID PROCEDURE, CLASSIFICATION OF CARCINOMA, SITE OF METASTASES, TOTAL DOSE OF I^{131} WITH RESPONSE AND COMPLICATIONS THEREOF, AND STATUS OF 21 PATIENTS WITH THYROID CARCINOMA

Patient				Rx prior to admission	Surgical thyroid procedures at MGH	Histologic diagnosis	Metastases
Case No.	Name; MGH No.	Age (yrs.)	Sex				
1	L. A. 677301	34	M	Laminect.; x-ray spine, 5,200 r; N mustard	Tot. thyroidect.	Adenocarcinoma arising in embryonal adenoma	Clavicle, pelvis, ribs, spine, skull, lungs
2	C.D. 676977	53	F	Partial thyroidect., 8 yrs.	Tot. thyroidect.	Adenocarcinoma arising in embryonal adenoma	(Local neck recurrence)
3	M.J. 752641	60	F	Biopsy cerv. lymph node	Tot. thyroidect.	Adenocarcinoma arising in embryonal adenoma	Lumbar vert., pelvis
4	A.M. 757026	55	F	Partial thyroidect.; x-ray neck, 6 yrs.	Tot. thyroidect.	Adenocarcinoma, Hürthle-cell type	Pelvis
5	M.S. 683429	60	F	None	Tot. thyroidect.	Adenocarcinoma arising in embryonal adenoma	Pelvis, clavicle
6	L.V. 706909	38	F	Partial thyroidect., 16 yrs. Biopsy, pelvic lesion, 2 mos.	Tot. thyroidect.	Adenocarcinoma arising in embryonal adenoma	Pelvis
7	J.B. 84196	68	M	None	Partial thyroidect., 1935. Tot. thyroidect., 1945	Adenocarcinoma arising in fetal adenoma	Lumbar vert., pelvis
8	T.I. 481114	43	F	Partial thyroidect., 11 yrs.	Tot. thyroidect.	Adenocarcinoma arising in fetal adenoma	Skull, lungs, cerv. vert.
9	E.N. 709264	77	F	None	Tot. thyroidect.	Adenocarcinoma arising in fetal adenoma	Clavicle, pelvis, lungs
10	A.W. 724362	62	F	Partial thyroidect.; x-ray neck, 18 mos.	Tot. thyroidect.	Adenocarcinoma arising in fetal adenoma	Skull
11	M.J. 790455	73	F	None	Tot. thyroidect.	Follicular adenocarcinoma	Clavicle, ribs
12	M.M. 624607	38	F	None	Tot. thyroidect.	Follicular adenocarcinoma	Lungs, ribs, pelvis, vert., humerus
13	M.P. 80959	82	F	Partial thyroidect.†; x-ray neck, 3,000 r, 4 yrs.	Biopsy of pelvic metast.	Follicular adenocarcinoma	Pelvis, skull, mandible, lumbar spine, lungs
14	N.S. 758220	50	M	Total thyroidect.; x-ray neck, 1 yr.	—	Follicular adenocarcinoma	Sternum, lungs, thoracic vert.
15	L.W. 808797	78	F	None	Thyroid biopsy	Follicular adenocarcinoma	Skull
16	L.D. 758213	24	F	Partial thyroidect., 7 yrs.; excis. local recur., 5 yrs.	Tot. thyroidect.	Mixed follicular & papillary adenocarcinoma	Lungs
17	M.D. 744775	14	F	Biopsy cerv. node, 1 mo.	Partial thyroidect. (inop.). Tot. thyroidect. with I^{131}	Mixed follicular & papillary adenocarcinoma	Lungs
18	R.C. 371857	16	F	None	Partial thyroidect. Tot. thyroidect. with I^{131}	Mixed follicular & papillary adenocarcinoma	Cerv. lymph nodes
19	E.L. 365865	25	F	None	Partial thyroidect.	Mixed follicular & papillary adenocarcinoma	Lungs
20	A.S. 827955	78	M	None	Thyroid biopsy	Papillary adenocarcinoma	Neck, axilla, pelvis
21	G.D. 687994	32	F	Partial thyroidect., 3 yrs.; x-ray neck, 3,000 r	Tot. thyroidect.	Hürthle-cell adenocarcinoma	Skull, lungs, clavicle, axilla, lumbar vert.

Total dose I^{131} mc.)	Response of metastases to I^{131}	Complics. of I^{131} therapy	Other Rx besides I^{131}	Status*
1,024	Bone pain relief	None	None	Dead, 27 mos.
127	Myxedema after I^{131} therapy	None	Thyroid for myxedema, 5 yrs.	Living; no recurr., 5 yrs.
692	Bone pain relief	None	Testosterone (Fig. 13)	Living with disease, 4 yrs.
647	Bone pain relief	None	Thyroid, 2 yrs.	Living with disease, 4 yrs. Recent path. fract. femur
1,312	Retarded growth of metast.; bone pain relief	Hematop. changes (Fig. 9). Ur. bladder (Table 3)	X-ray pelvis, 3,000 r; thyroid. (Fig. 11)	Living with disease, 5 yrs.
216	Bone pain relief	Cessation of menses (Table 3)	Thyroid; x-ray pelvis	Living with disease, 5 yrs.
1,275	Retarded growth of metast.	None	X-ray spine, 1,500 r in 1945; 3,000 r in 1954; laminect., 1945. Thyroid, 2 yrs.	Living with disease, 10 yrs. after tot. thyroidect.; 8 yrs. after 1st I^{131}
503	Part. control of growth of metast.	None	X-ray skull, 1,500 r	Dead, 7 yrs.
None	—	—	X-ray clavicle, 1,800 r	Dead, 3 yrs.
62	Slight decr. in size of metast.	None	Excis. skull metast., 3 mos. after tot. thyroidect.; 2 mos. after I^{131}	Living 4 yrs. with ? new skull metast.
816	Decr. in size of rib metast.	None	Excis. clav. metast. at time of thyroidect. Thyroid, 18 mos. (Fig. 12)	Living with disease, 3 yrs.
2,000	Bone pain relief; control of some metast. (Figs. 7, 8)	Cessation of menses (Table 3); elev. BSP; ? pancytopenia	None	Dead, 5½ yrs. after tot. thyroidect. & 5 yrs. after 1st I^{131}
360	Bone pain relief	None	None	Dead, 6 yrs. after part. thyroidect. & 2 yrs. after 1st I^{131}; failed to return for I^{131}
500	None	None	Excis. sternal metast. (Fig. 4)	Living with disease, 3 yrs.
—	—	—	Testost. prop., 50 mg. 3× wk., for 3 mos.	Dead, 1 yr.
300	Disapp. of metast. by roentg'm.	None	Thyroid, 3 yrs.	Living with ? recurr. in lungs, 4 yrs.
500	Slight decr. in pulm. metast.	None	X-ray lungs, 2,200 r. Testost., 2 mos.	Dead, 20 mos.
70	—	—	None	Living with no evident disease, 7 yrs.
140	Disappear. of pulm. lesions by roentg'm.	None	X-ray neck, postop. Thyroid, 9 yrs.	Living with no recurr.
—	—	—	X-ray neck, 5,000 r. Thyroid, 5 mos. Testost., 6 mos.	Dead, 11 mos.
—	—	—	Thyroid, 6 yrs. X-ray; 1,400 r skull; 7,050 r, back; 3,600 r, chest; 2,400 r, shoulder	Living with diffuse metast.

* Period following surgical procedure at MGH and following initiation of I^{131} therapy in most patients.
† Probably total thyroidectomy in view of subsequent x-ray therapy and failure to detect significant I^{131} in thyroid area.

administered in an attempt to destroy or to inhibit the growth of metastases. This report will attempt to evaluate this therapeutic program and will include an appraisal of the following:

(a) The use of total thyroidectomy and the antithyroid drugs for the purpose of stimulating the metastases in an attempt to increase their avidity for I^{131}.

(b) The functional and histologic variations in metastases in the same patient.

(c) The general bodily effects of the administration of large doses of I^{131}.

Due to the restricted usefulness of I^{131} therapy, desiccated thyroid and testosterone were given to some patients in an attempt to inhibit the growth of metastases by depressing pituitary activity.

FACTORS INFLUENCING I^{131} UPTAKE IN METASTATIC THYROID CARCINOMA

This survey comprises observations on a group of 21 selected patients with metastatic thyroid carcinoma. A summary of the data is included in Table 1. The primary tumor was classified as adenocarcinoma arising in an adenoma in 10 patients; as follicular adenocarcinoma in 5; as mixed papillary and follicular in 4; as pure papillary in 1; and as Hürthle-cell adenocarcinoma in 1.

Total thyroidectomy

A program of attempting to induce function in thyroid metastases by total thyroidectomy was first suggested by Means (4) in 1937 and undertaken by Rawson *et al.* (1) in 1948. Such a program was carried out inadvertently by Von Eiselsberg (5) in 1894. He reported the development of transient symptoms of myxedema in a woman 38 years of age, following total thyroidectomy for thyroid carcinoma. Apparent alleviation of the myxedema coincided with growth of a metastatic lesion in the manubrium, which may have acquired function as a result of the total thyroidectomy. Surgical total thyroidectomy is preferable to radiation thyroidectomy, because (a) thyroidectomy is usually complete, (b) the desired effect is attained rapidly, and (c) the parent thyroid tumor is visualized and its function can be assayed.

Surgical total thyroidectomy was performed in 15 of the 21 patients. Following this procedure, an uptake of I^{131} was induced in the metastases of 7 of the 15 and was increased in the metastases of 5 other patients (Table 2). A concomitant growth stimulus was imparted to the metastases, since growth of the lesions was evident by roentgenograms in 14 of the 15 patients. Growth of a metastasis following total thyroidectomy is exemplified by the case of a 34-year-old male (L. A., Case 1) with widespread

metastases who became paraplegic four days following total thyroidectomy. He had been semi-ambulatory with known metastases for nine years prior to this procedure. Fourteen days after the total thyroidectomy there was an appreciable uptake of I^{131} in a vertebral metastasis and treatment with I^{131} was initiated. Aggravation of the symptoms and signs of spinal cord compression occurred thirty-four days following total thyroid-

TABLE 2. EFFECT OF TOTAL THYROIDECTOMY AND ADMINISTRATION OF THE ANTITHYROID DRUGS ON THE GROWTH AND UPTAKE OF I^{131} BY THE METASTASES OF PATIENTS WITH THYROID CARCINOMA

Case No.	Total thyroidectomy			Antithyroid drugs	
	Growth of metast.	Induced I^{131} uptake	Increased I^{131} uptake	Growth of metast.	Increased I^{131} uptake
1	+		+	?	+
2	+	+		?	+
3	+	+		+	0
4	+	+		+	0
5	+	+		+	+
6	+	+		0	+
7	0		+	+	+
8	+		+	+	+
9	+	?			
10	+		+		
11	+	+		+	+
12	+	+		+	+
14	+	?		+	?
16	+		+	?	?
17				?	?
21	+	0		+	0

+ = positive effect.
0 = no effect.
? = not evaluated.

ectomy in another patient (H.O'B, MGH #547221) with a vertebral metastasis. Residual iodine-containing contrast medium from a previous myelogram prevented treatment with I^{131}.

It is difficult to make an accurate appraisal of the growth rate of thyroid metastases following total thyroidectomy, since the subsequent course of the disease is usually complicated by various therapeutic measures. The sequence of events in the foregoing 2 patients is a dramatic and exaggerated portrayal of the effect of total thyroidectomy on the growth of metastases.

Nevertheless it emphasizes the potentiality of this procedure and the need of bearing in mind the resulting growth stimulus in attempting to increase the uptake of I^{131}.

Antithyroid drugs

The antithyroid drugs are also effective in increasing the uptake of I^{131} by metastases from thyroid carcinoma; however, these drugs may also stimulate growth of the tumor. The administration of antithyroid drugs to 14 of the 21 patients increased the uptake of I^{131} in the metastases of 8

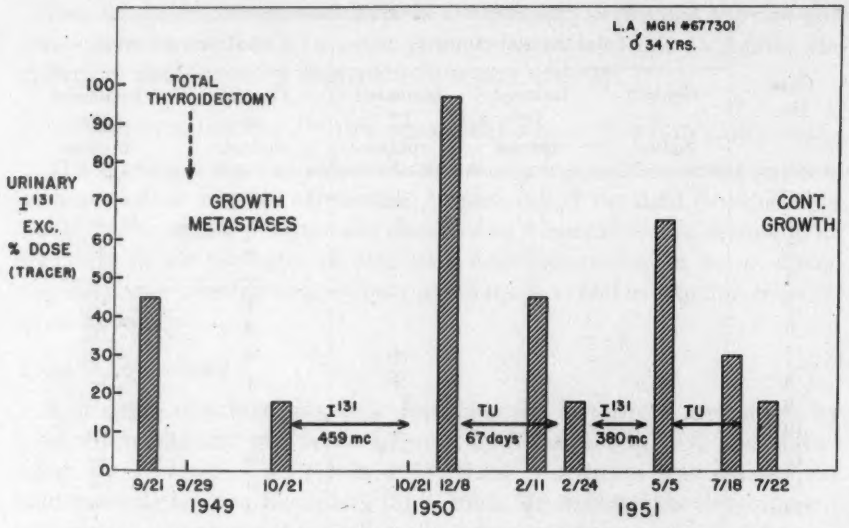

FIG. 1. The continued effectiveness of thiouracil administration in increasing the uptake of I^{131} by thyroid metastases, even after therapy with 839 mc. of I^{131}

patients (Table 2). Growth of metastatic lesions was evident by roentgenograms in 9 of the 14 patients; in 3 of the 9 patients this growth was unaccompanied by a significant increase in the uptake of I^{131} by the metastases. In 1 patient an increased uptake of I^{131} was unaccompanied by evident growth of metastases. In Patient L.A. (Case 1) thiouracil continued to be effective in increasing the uptake[1] of I^{131} by the metastasis even after treatment with 839 mc. of I^{131} (Fig. 1). However, the lesion grew relentlessly and he died in spite of treatment with 1,024 mc. of I^{131} over a 27-month period. In this patient the growth stimulus provided to the metas-

[1] This assumed uptake is derived from subtracting the percentage of the 48-hour urinary excretion of a tracer dose of I^{131} from 100 per cent. This holds for subsequent similar observations.

tases by the antithyroid drugs may have exceeded the benefit derived from increasing the uptake of I^{131}. In Patient J.B. (Case 7) the administration of thiouracil did not continue to be effective in increasing the uptake of I^{131} by small vertebral and iliac metastases after treatment with 600 mc. of I^{131} (Fig. 2), although this treatment had apparently controlled the growth of these lesions over a four-year period. A subsequent course of 1-methyl 2 mercaptoimidazole (Tapazole) in a dosage of 15 mg. every eight hours by mouth for three months failed to increase the uptake of

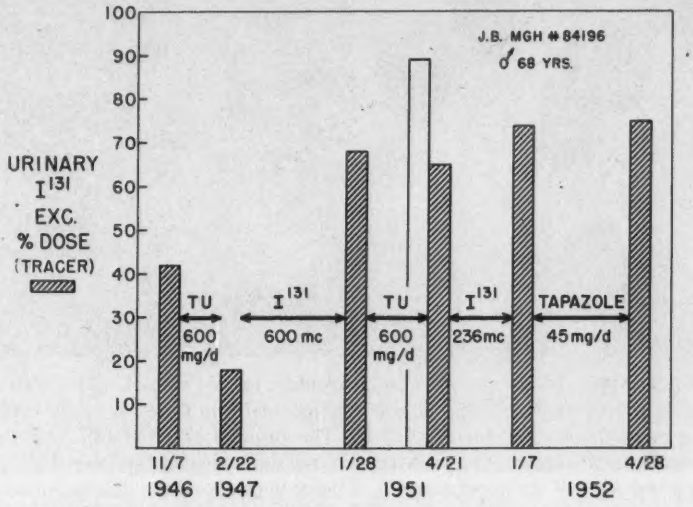

FIG. 2. The failure of thiouracil administration to increase the uptake of I^{131} by thyroid metastases after therapy with 600 mc. of I^{131}.

I^{131}. In such a patient, repeated unsuccessful attempts to increase the uptake of I^{131} in the metastases by the antithyroid drugs may only serve to stimulate growth of these lesions. Tissue obtained from the vertebral metastasis eight years following the initiation of I^{131} therapy (total dose 1,275 mc.) revealed persistent tumor without evident radiation effect.

Variations in function and structure of metastases

Other factors which influence the response to I^{131} treatment are the variations in function and histologic pattern of metastases in the same patient.

Single metastases. Differences in function between parts of a single metastasis are illustrated in the following study. Patient N.S. (Case 14), a

50-year-old white male, had noted a mass in the manubrium for one year. Six months prior to his entry to the MGH total thyroidectomy had been performed and a diagnosis of thyroid carcinoma had been made. After preliminary studies, he was given 200 millicuries of I^{131}, of which he excreted 90 per cent in the urine in seventy-two hours. Ten days later a needle biopsy specimen of the sternal mass was obtained. In comparing parts of this minute piece of tissue, a marked difference was observed in the

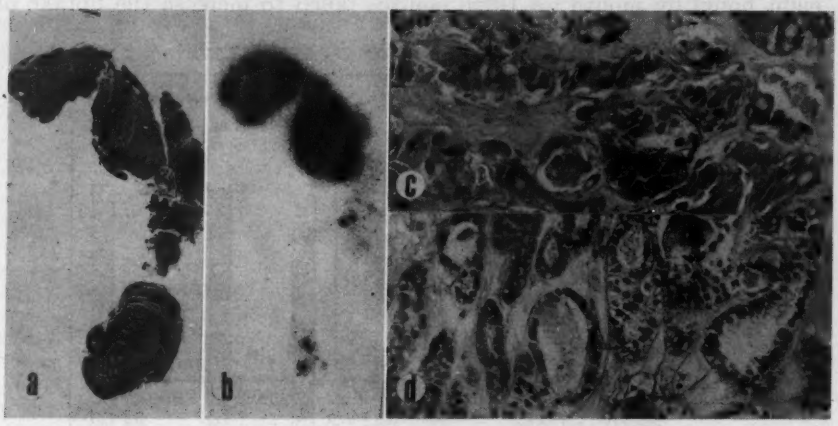

FIG. 3. Needle biopsy specimen from a solitary sternal metastasis ten days following the administration of an initial dose of 200 mc. of I^{131} in Case 14. **(a)** Low-power view of the needle biopsy specimen (×15). **(b)** The autoradiograph of **(a)**. Note the marked blackening in the upper segment with minimal darkening in the lower half (×15). **(c)** High-power view of the upper segment of tissue in **(a)**. Note the disarray of acinar architecture, nuclear atypicalities, cytoplasmic swelling and interstitial edema—attributable to radiation damage (×170). **(d)** High-power view of the lower biopsy fragment, showing good follicular differentiation. The scant darkening of the autoradiograph, as noted in the lower half of **(b)**, is consistent with the conspicuous absence of significant radiation effect. Contrast with **(c)** (×170).

I^{131} content and the effects of radiation (Fig. 3 a, b, c, d). This was particularly impressive in view of the excellent differentiation in the areas which failed to concentrate the isotope. Three months after the treatment with 200 mc. of I^{131}, this sternal metastasis was excised. Some areas showed a marked radiation effect, others showed little or none (Fig. 4 a, b). This variation in I^{131} uptake in a metastasis was observed in other patients in whom single doses of I^{131} as large as 350 mc. failed to destroy any solitary osseous metastasis. The phenomenon of the variation in I^{131} uptake in a metastasis suggests that portions of a tumor are more active than others at any one time and may explain why a lesion would be only partially vulnerable to a single dose of I^{131}.

Serum PBI level: An interesting observation will be presented that is compatible with the idea of fractional concentration of I^{131} in metastases at any one time. It concerns the change in the serum protein-bound iodine (PBI) level following the administration of three therapeutic doses of I^{131} to Patient M.P. (Case 13). This 82-year-old woman had a right total and left subtotal thyroidectomy because of an adenocarcinoma of the

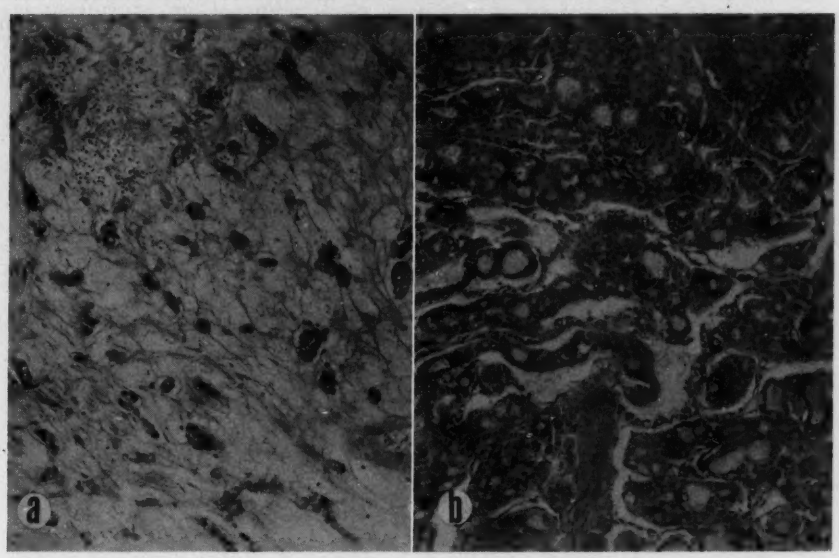

FIG. 4. Photomicrographs of representative areas of the sternal metastasis (Case 14) removed three months following the administration of an initial dose of 200 mc. of I^{131}. Note the marked variation in the effects of radiation. **(a)** Zone of marked radiation damage with fibrosis, edema, and scattered distorted follicles (×100). **(b)** Region with little radiation effect, as shown by preservation of follicular architecture and minimal signs of fibrosis and cellular abnormalities (×100).

thyroid. Four years later, biopsy of a mass in the mandible revealed well differentiated thyroid carcinoma and she was referred to the MGH for consideration of I^{131} therapy. Roentgenograms showed pulmonary metastases and osteolytic metastases in the skull, mandible, pelvis and lumbar spine. Since the metastases in the mandible, pelvis and spine concentrated and retained I^{131}, she was given 60 mc. of the isotope. The serum PBI level rose from 6.2 micrograms to 28.0[2] micrograms per 100 ml. in seven days (Fig. 5). In two months, a second dose of I^{131} (100 mc.) was given; there was no change in the serum PBI level. Two months following the second

[2] There was no evidence of hypermetabolism at this time.

dose, she was given 200 mc. of I^{131}. The serum PBI level rose from 7.1 micrograms to 12.4 micrograms per 100 ml. in eleven days. The increase in the serum PBI concentration in eleven days was similar to the gradual increase in the serum PBI^{131} concentration in this patient previously noted by Stanbury *et al.* (6).

The rise in the level of serum PBI following the first dose of I^{131} (60 mc.) was probably due to damage of tissue in the metastases which concentrated I^{131} (7). The 78 per cent uptake of the second dose of I^{131} (100 mc.), given

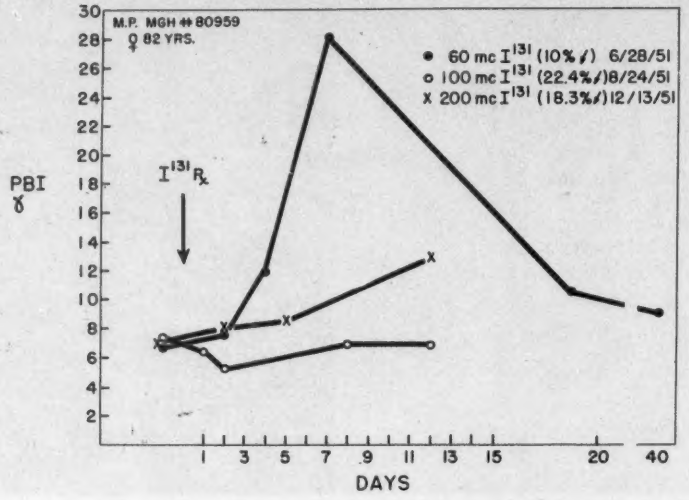

FIG. 5. The level of PBI (γ/100 ml.) in the serum of Patient M. P. (Case 13) before and after treatment with I^{131}. (*See* text for details.)

two months later, is evidence of residual functioning tumor tissue. The failure of the serum PBI level to rise following this second dose of I^{131} suggests that the isotope became concentrated in previously damaged focal areas which had been depleted of thyroglobulin, or became concentrated in other areas but did not damage tissue. The rise in the serum PBI level following the third dose of I^{131} (200 mc.) suggests that further damage occurred in the initially irradiated areas which had now replenished their store of thyroglobulin, or that damage occurred in new areas which contained thyroglobulin. The latter explanation would be compatible with the idea of the fractional concentration of I^{131} in metastases at any one time. This increase in the concentration of serum PBI following a relatively small initial dose of I^{131} to a patient with extensive functioning metastases was also noted in Patient M.M. (Case 12).

Effect of antithyroid drugs on PBI level: Another observation referable

to the idea of the focal avidity for iodine in a metastasis is the effect of thiouracil administration on the serum PBI level in Patient M.S. (Case 5). This 60-year-old woman entered the MGH in 1949 complaining of pain in the right hip of one year's duration. Roentgenograms revealed a large osteolytic lesion in the right pelvis. A biopsy specimen showed well differentiated adenocarcinoma of thyroid origin. A total thyroidectomy was performed in December 1949, resulting in an increase in the concentration of I^{131} by the metastasis. Over the next three months she received

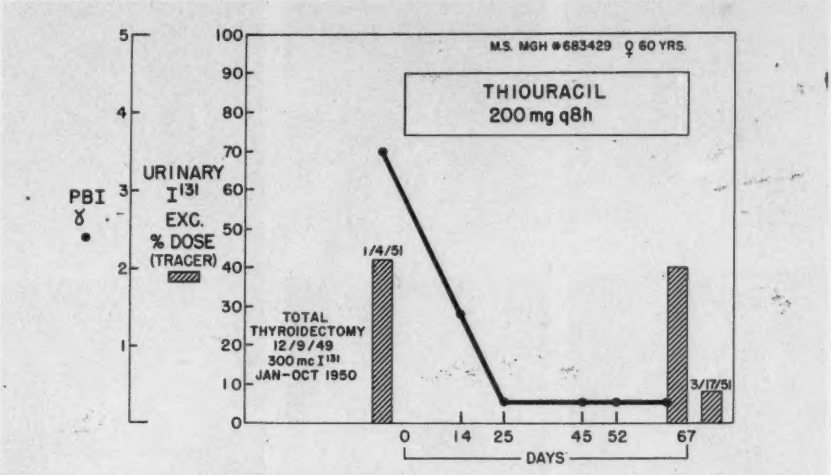

FIG. 6. The effect of the oral administration of thiouracil (200 mg. every eight hours for sixty-seven days) on the serum PBI level (γ/100 ml.) and the 48-hour urinary excretion of a tracer dose of I^{131} in Patient M.S. (Case 5). (*See* text for details.)

300 mc. of I^{131}; there was marked relief of bone pain, but no change in the appearance of the tumor by roentgenogram. In January 1951 she was given thiouracil for sixty-seven days in an attempt to increase the uptake of I^{131} in the pelvic lesion. In Figure 6 it may be noted that the oral administration of thiouracil (200 mg. every eight hours) lowered the serum PBI level from 3.4 to 0.2 microgram per 100 ml. in twenty-five days. The urinary excretion of 9 per cent, three days after thiouracil was discontinued, is evidence that the uptake of I^{131} in the pelvic metastasis was increased.

The lowering of the serum PBI concentration to 0.2 microgram per 100 ml. in twenty-five days suggests that this large tumor mass contained only a small store of thyroid hormone. Histologic analysis of biopsy material from this metastasis showed that colloid was only focally distributed in the follicular adenocarcinoma; hence the small store of thyroid hormone was probably depleted from only a portion of the tumor.

Structure of different metastases in same patient: The functional variations existing in a single metastasis have been described and appear to limit the vulnerability of a metastasis to a single dose of I^{131}. Histologic variations occur in different metastases in the same patient, and also limit the response to I^{131}. Such histologic variations were noted in 6 patients (Cases 1, 4, 5, 8, 11 and 12), and are illustrated by observations in Patient M.M. (Case 12). This 38-year-old woman had a total thyroidectomy

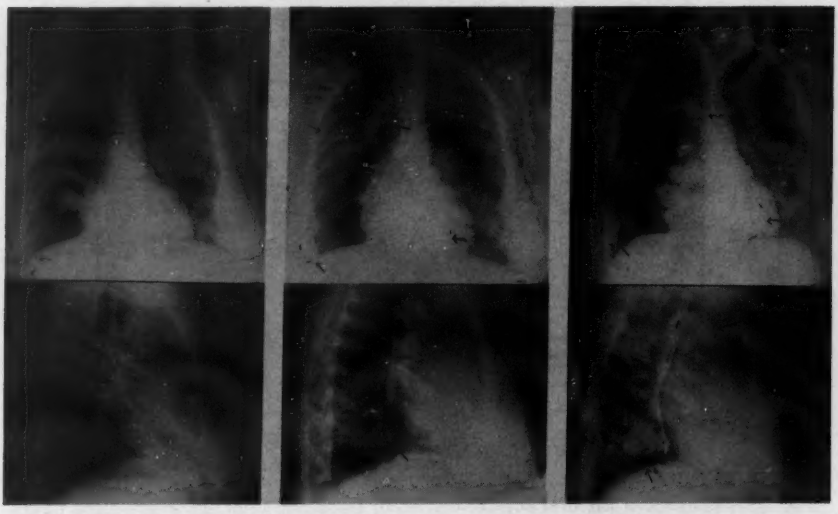

FIG. 7. Roentgenographic appearance of pulmonary and skeletal metastases of Patient M. M. (Case 12) in response to I^{131} therapy. Growth of the rib metastasis (seventh rib on right) was controlled, whereas the pulmonary metastases continued to grow. The total doses of I^{131} administered up to the time of each roentgenogram are noted in the upper left-hand corner. (*See* Figure 8 for histologic picture.)

in 1948 for an adenocarcinoma of the thyroid. Six months following this procedure she sustained a pathologic fracture of the left humerus and noted severe pain in the left hip. Roentgenograms revealed pulmonary metastases and osteolytic lesions in the ribs, humerus, pelvis and lumbar vertebrae. Some of these lesions concentrated I^{131} and over the next five years she was given a total dose of 2,000 mc. of I^{131}. Roentgenograms of the chest revealed that growth of the lesion in the seventh right rib was controlled, whereas the pulmonary lesions continued to grow (Fig. 7). At autopsy, the metastasis in the seventh right rib showed a marked radiation effect, whereas the pulmonary lesions showed poor histologic differentiation and no radiation damage (Fig. 8a and b). Similar variations in

histologic differentiation and effects of radiation were noted in the pelvic metastases. Such poorly differentiated areas, if accessible to surgery, should be excised in view of their poor response to I^{131} therapy.

GENERAL BODILY EFFECTS OF LARGE DOSES OF I^{131}

To assess the possible general bodily effects of the administration of large doses of I^{131}, an evaluation was made of pituitary, adrenal, gonadal, renal and hepatic function in some patients (Table 3). Changes in the

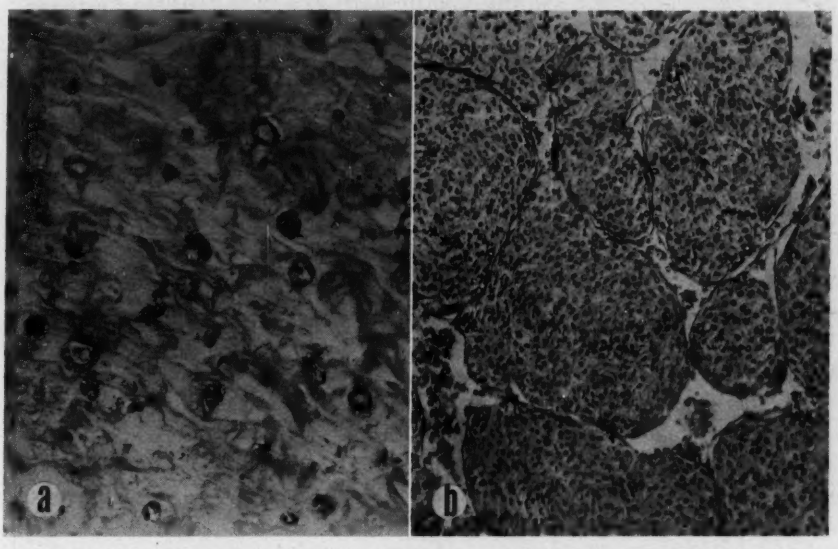

FIG. 8. Photomicrographs of tissue obtained at autopsy from Patient M. M. (Case 12) after the administration of a total dose of 2,000 mc. of I^{131} over a five-year period. **(a)** Tissue from the right seventh rib, showing severe radiation damage (×140). **(b)** Tissue from the pulmonary metastases, showing lack of differentiation and no radiation effect (×140).

hematopoietic system were also followed. To date, complications of I^{131} therapy in patients with metastatic thyroid carcinoma have been minor in nature, considering the large doses of the isotope that have been given.

Pituitary: The level of follicle-stimulating hormone (FSH) in the urine (8) was followed in 4 women aged 60, 60, 73 and 38 years (Cases 3, 5, 11 and 12), and 1 man aged 68 years (Case 7). Although iodine is known to concentrate in the pituitary (9–12), no alteration in this assay of pituitary function was noted after the administration of total doses of 692 to 2,000 mc. of I^{131} extending over periods of from three to eight years.

Adrenals: The level of 17-ketosteroids in the urine (13) as an assay of

adrenal function was followed in 3 women aged 60, 38 and 16 years (Cases 5, 12 and 18). No changes were noted after the administration of total doses of 70 to 2,000 mc. of I^{131} extending over periods of from two to five years.

Gonads: Menstruation, which had been normal, stopped permanently after the administration of total doses of 50 mc. and 131 mc. of I^{131} to Patients L.V. (Case 6) and M.M. (Case 12). These women were both

TABLE 3. EFFECTS OF I^{131} THERAPY ON PITUITARY, ADRENAL, GONADAL, HEPATIC AND RENAL FUNCTION AND THE HEMATOPOIETIC SYSTEM IN PATIENTS WITH METASTATIC THYROID CARCINOMA

	NO. PTS.	STUDIES	DOSE I^{131} (mc)	EFFECT	COMMENT
PITUITARY	5	FSH	692–2000	None	
ADRENALS	3	17KS	70–2000	None	
GONADS	5	Catamenia	50, 131 70, 300 140	2 Stopped Cta 2 None 1 None	Large Pelvic Mass No Pelvic Lesion 2 Subsequent Pregnancies
LIVER	3	BSP TPr Ceph Flocc Alk Phosp.	721 – 2000	1 ↑ BSP None	
KIDNEY	4	Urinalysis Urea cl. PSP UCT NPN Cystoscopy	503–2000	None Reddening of Bladder	Tumor in Proximity to Bladder
BLOOD	17	WBC and Smear Platelets Hb Bleeding Time Bone Marrow	27–2000	↓ WBC ↓ Platelets ↓ Hb ↓ Response to Fe Rx ↑ Bleeding Time Erythroid Hyperplasia	Reversible ?

38 years of age, and had large osseous pelvic metastases which concentrated I^{131}. Patient L.V. received 50 mc. of I^{131}, of which 82 per cent was excreted in the urine in forty-eight hours; menses failed to appear after this dose. Patient M.M. received 3 doses of I^{131} totalling 131 mc. over a three-month period. Menstruation failed to appear following this course of therapy and the level of FSH in the urine was consistent with that of a patient with ovarian failure. She died after receiving a total dose of 2,000

mc. of I^{131} over a five-year period. At autopsy the ovaries showed fibrosis and cortical atrophy.

In 2 women (Cases 16 and 18) with no pelvic metastases, total doses of 300 and 70 mc. of I^{131}, respectively, failed to disturb their menses. Another woman, E.L. (Case 19), 25 years of age, with only pulmonary metastases received a total dose of 140 mc. of I^{131} over a period of twelve months. Subsequently she had two normal pregnancies and both children are living and well (2 and 5 years of age).

Hepatic function: In 3 patients (Cases 1, 7 and 12) serial observations of hepatic function were made over a period of two to seven years after the administration of total doses ranging from 1,024 to 2,000 mc. of I^{131}. These tests included determinations on serum of total protein (A/G ratio), cephalin flocculation, alkaline phosphatase, and retention of bromsulphalein (BSP) dye. The only abnormality noted was in the 45-minute retention of BSP in the serum. A rise in the serum BSP retention to 24 per cent four days after the administration of 166 mc. of I^{131} occurred in Patient M.M. (Case 12), who had previously received a total dose of 555 mc. of I^{131}. Over the next two years, the elevated retention of BSP in the serum persisted. The patient died after receiving a total dose of 2,000 mc. of I^{131} over a five-year period. At postmortem examination the liver showed a mild degree of fatty change.

Renal function: Four patients (Cases 1, 7, 8 and 12) had serial observations extending over periods of from two to seven years during which total doses of 503 and 2,000 mc. of I^{131} were administered. These observations included determination of serum nonprotein nitrogen and urea clearance, urinalysis, urine concentration test, and phenolsulfonphthalein test. No abnormalities were noted.

Following the administration of 80 mc. of I^{131}, reddening of the right half of the urinary bladder occurred in Patient M.S. (Case 5), who had a tumor mass which concentrated I^{131} in that side of the pelvis. This observation was reported previously in 1951 (3). Follow-up cystoscopic studies have revealed no abnormality in the urinary bladder to date.

Hematologic changes: White blood cell, differential, and reticulocyte counts, and hemoglobin level were determined in all patients following the administration of therapeutic doses of I^{131}. A platelet count and bone marrow analysis were occasionally made. Following I^{131} therapy a transient decrease occurred in the number of circulating white blood cells, especially the lymphocytes. Apparently, the decrease in the lymphocytes is not related to the magnitude of the dose of I^{131}, since it was noted in Patient M.M. (Case 12) after the administration of the first dose of only 27 mc. of I^{131} (41 per cent excreted in the urine in forty-eight hours). The administration of subsequent single doses up to 320 mc. of I^{131} to this pa-

tient caused no greater decrease in the number of circulating lymphocytes than the smaller dose.

Following I^{131} therapy, the blood hemoglobin level decreased only in Patient M.M. (Case 12). After having been given a total dose of 1,700 mc. of I^{131} over a five-year period, she was given 300 mc. of I^{131}, of which she excreted 40 per cent in the urine in forty-eight hours. A gradual lowering of the level of hemoglobin in the blood from 10.0 grams to 6.0 grams per 100 ml., and of the white blood cells from 5,200 to 2,400 per cu. mm. occurred in the next two months. The oral administration of ferrous sulfate failed to alter the lowering of the hemoglobin level. The patient died at home at this time and cutaneous ecchymoses were noted at autopsy. Complete blood indices were unavailable, but it would appear that she had a peripheral pancytopenia. The bone marrow, at autopsy, appeared to be normal or even hypercellular. Two patients had low hemoglobin levels prior to I^{131} irradiation. The administration of ferrous sulfate by mouth failed to raise the low hemoglobin levels of these 2 patients during periods of four to six months following the administration of single doses of 200 and 300 mc. of I^{131}.

In Patient M.S. (Case 5) skin ecchymoses developed after administration of a total of 1,077 mc. of I^{131} over a period of three years (Fig. 9). The white blood cell count had decreased gradually from 8,000 to 4,000 per cu. mm. in this interval. Blood studies showed positive results with the tourniquet test, a decreased number of white blood cells with a normal differential count, decreased platelets, and a normal bone marrow.[3] External radiation (3,000 r) to the pelvic lesion and 235 mc. of I^{131} (47.9 per cent excreted in the urine in forty-eight hours) were administered in the next six months. An increased bleeding time and erythroid hyperplasia in the bone marrow, despite a normal level of hemoglobin in the blood, were then noted. Further I^{131} therapy was withheld, and treatment with desiccated thyroid (3 grains daily, by mouth) was instituted in an attempt to prevent growth of the metastasis. Eighteen months later, results of the tourniquet test, the platelet count and the bleeding time were normal. The low white-blood cell count and the erythroid hyperplasia in the bone marrow persisted. Thus, during the foregoing regimen, some of the abnormal hematologic findings returned to normal.

Concerning I^{131} irradiation and hematologic changes, the following case history seems worthy of note. Patient M.J. (Case 11) entered the MGH in 1952 complaining of a painless lump in the neck, of two years' duration. Roentgenograms revealed osteolytic lesions in the medial aspect of the right clavicle and the left sixth rib. Biopsy of the rib lesion showed a well differentiated adenocarcinoma of thyroid origin. A total thyroidectomy,

[3] We are indebted to Dr. Bernard Jacobson for hematologic observations.

as well as excision of the clavicular lesion, was performed in October 1952. Four months following this procedure the patient was started on 1-methyl-2-mercaptoimidazole (Tapazole), 15 mg. by mouth every six hours, in an attempt to increase the uptake of I^{131} in her metastases. After the administration of this antithyroid drug for two months, agranulocytosis developed. For treatment of this complication, cortisone was administered

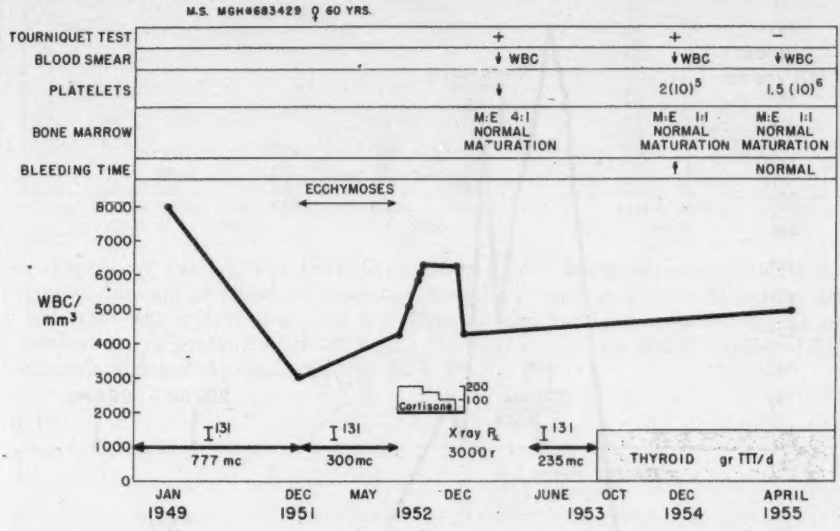

FIG. 9. Gradual lowering of the white blood cell count, ecchymoses, positive response to the tourniquet test, and a low platelet count in Patient M. S. (Case 5) following the administration of a total dose of 1,077 mc. of I^{131} over a three-year period. Increased bleeding time and erythroid hyperplasia in the bone marrow were subsequently noted following 3,000 r of external radiation and the administration of 235 mc. of I^{131}. The tourniquet-test response, the platelet count, and the bleeding time became normal eighteen months following the cessation of radiation therapy.

for fourteen days (Fig. 10). The white blood cell count rose from 500 to 44,000 per cu. mm. on the eighth day of cortisone therapy and then decreased to normal over the next six days. In spite of the administration of a therapeutic dose of 300 mc. of I^{131} and two additional doses of I^{131} totalling 516 mc. over the next year, the white count did not fall below normal.

INHIBITION OF GROWTH OF METASTASES BY DEPRESSING PITUITARY ACTIVITY

The restricted usefulness of I^{131} in the treatment of patients with metastatic thyroid carcinoma and the hematopoietic complications of therapy with this isotope in Patient M.S. (Case 5) led to a search for other

therapeutic agents. Desiccated thyroid and testosterone were administered in an attempt to inhibit the growth of metastases by depressing pituitary activity.

Desiccated thyroid was given to Patient M.S. (Case 5) in the hope of decreasing pituitary TSH activity and its potential effect on the growth

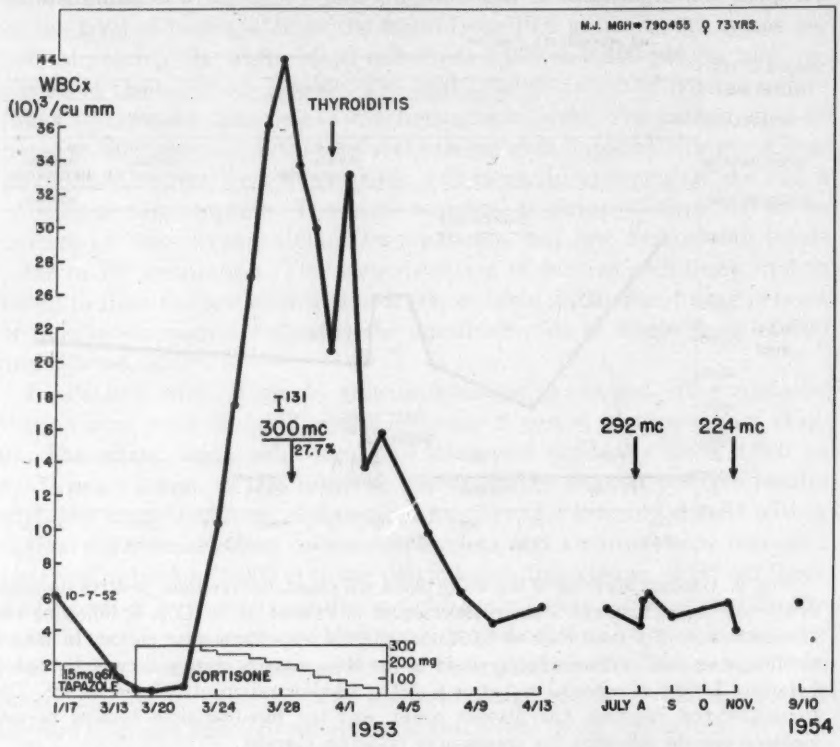

FIG. 10. In Patient M. J. (Case 11) agranulocytosis developed following the administration of 1-methyl-2-mercaptoimidazole, 15 mg. by mouth every six hours. Recovery took place coincident with the administration of cortisone for fourteen days. In spite of the administration of 300 mc. of I^{131} and two additional doses totalling 516 mc., the white blood cell count did not fall below normal.

of the metastases. This program seemed logical, since the metastatic lesion grew following total thyroidectomy and its function was inhibited by thiouracil, suggesting that the tumor was under the influence of the pituitary. By roentgenogram, the size of the pelvic metastasis remained stationary for eighteen months and clinically the patient was well (Fig. 11). She took 3 grains of desiccated thyroid per day by mouth. On this dose she excreted 90 per cent of a tracer dose of I^{131} in the urine in seventy-two hours. After

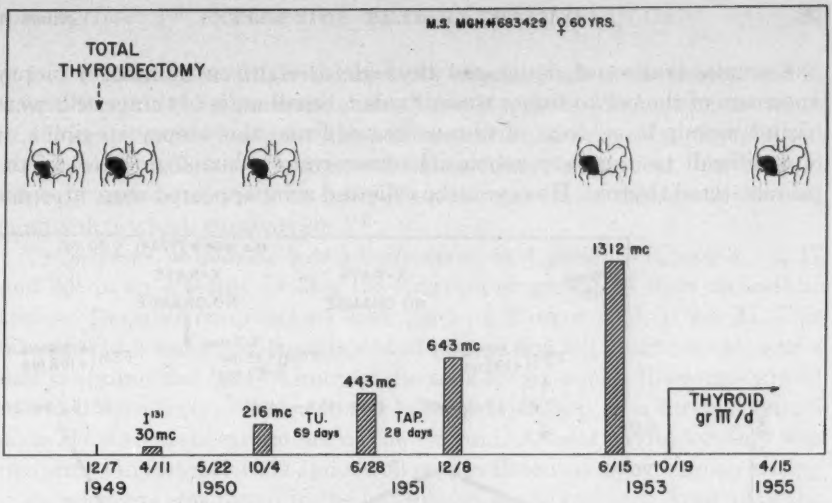

FIG. 11. In Patient M. S. (Case 5) growth of a pelvic metastasis was controlled during the oral administration of desiccated thyroid for eighteen months. Growth of the metastasis was evident after total thyroidectomy and had been controlled by the administration of a total dose of 1,312 mc. of I^{131} over a four-year period. (Cross-hatched columns represent the cumulative dosage of I^{131}.)

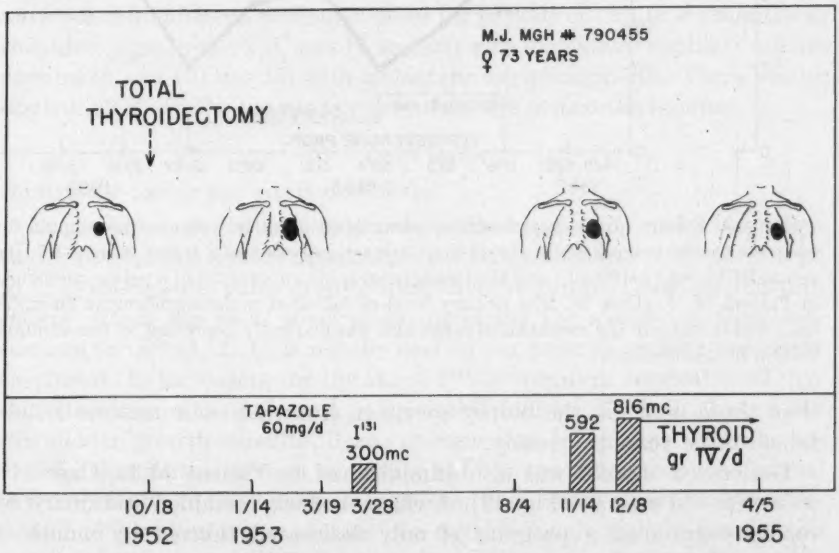

FIG. 12. Control of the growth of a rib metastasis for eighteen months during the administration of desiccated thyroid (Case 11). Growth of the lesion was evident after total thyroidectomy but the administration of a total dose of 816 mc. of I^{131} over the next year inhibited further growth prior to the administration of thyroid.

the administration of desiccated thyroid for eighteen months, a biopsy specimen of the pelvic tumor was obtained. Small nests of tumor cells were visible among large areas of fibrous tissue. From this biopsy specimen, it was difficult to draw any conclusions concerning a histologic effect of the administered thyroid. However, the cells and acini appeared more atrophic

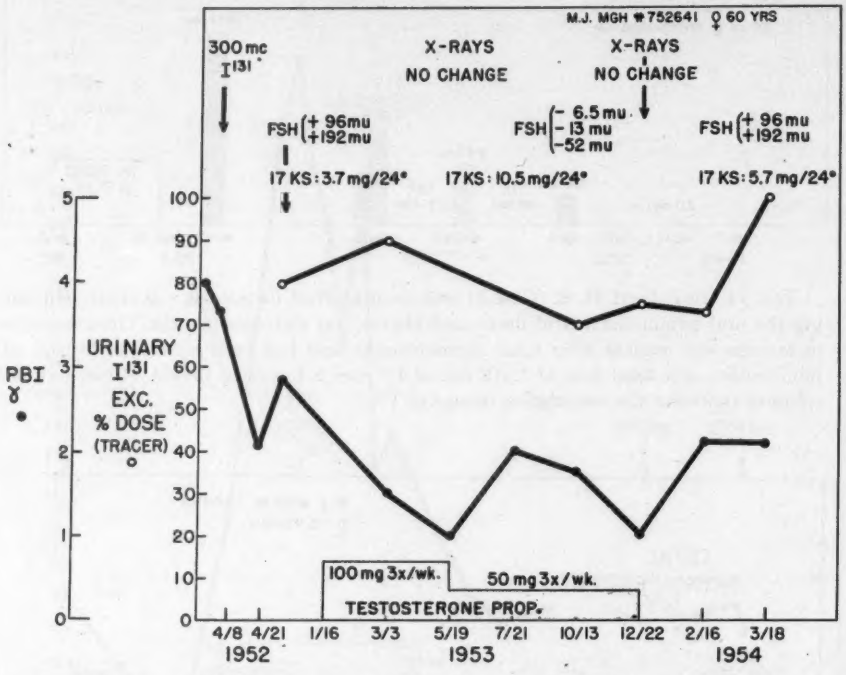

FIG. 13. Failure of the intramuscular administration of testosterone propionate for one year to effect significantly the 48-hour urinary excretion of a tracer dose of I^{131}, the serum PBI level (γ/100 ml.) and the roentgenographic appearance of a pelvic metastasis in Patient M. J. (Case 3). The urinary level of follicle-stimulating hormone (m.u./24 hrs.) was initially in the menopausal range and was markedly depressed by the administration of testosterone.

than those noted in the biopsy specimen from the same metastasis, obtained three years previously.

Desiccated thyroid was also administered to Patient M.J. (Case 11), a 73-year-old woman (Fig. 12). A rib metastasis remained stationary by roentgenogram on a program of only desiccated thyroid by mouth (4 grains daily) for eighteen months. Growth of the lesion was evident following total thyroidectomy and was inhibited by I^{131} therapy. Patients M.S. and M.J. (Cases 5 and 11) were euthyroid when desiccated thyroid was initiated.

In 1 patient (Case 20) with primarily papillary adenocarcinoma, and in 1 with Hürthle-cell adenocarcinoma (Case 21), there was no concentration of I^{131} in the metastases, and the administration of desiccated thyroid had no inhibitory effect on their growth. Hence it would appear that thyroid therapy may be of value in controlling the growth of metastatic thyroid carcinomas which concentrate I^{131}.

Testosterone propionate was administered to 4 patients (Cases 3, 15, 17 and 20) in an attempt to alter the function or growth of their metastatic lesions. Detailed observations were made in Patient M.J. (Case 3). This 60-year-old woman had complained of pain in the left thigh for two and a half years and had noted a lump in the neck for six weeks. Roentgenograms revealed an osteolytic lesion in the left pelvis. Biopsy of a cervical lymph node showed adenocarcinoma of the thyroid. A total thyroidectomy was performed in October 1951 and a well encapsulated adenocarcinoma arising in an adenoma was found in the left lobe of the thyroid. In April 1952 she was given 300 mc. of I^{131}, of which she excreted 97 per cent in the urine in forty-eight hours. Eight months later testosterone propionate was administered intramuscularly for twelve months (Fig. 13). No significant effect was noted on the function, growth and roentgenographic appearance of the pelvic metastasis, although pituitary activity, as measured by the level of FSH in the urine, was markedly depressed. Testosterone propionate was administered in similar doses for periods of two to six months to the other 3 patients—2 (Cases 17 and 20) with metastatic papillary adenocarcinoma and 1 (Case 15) with metastatic adenocarcinoma. There was no obvious effect on the function or growth of the metastatic lesions.

DISCUSSION

Radioactive iodine and antithyroid drugs

In general, it is thought that potentially functioning metastases from thyroid carcinoma neither accumulate nor retain enough I^{131} for the isotope to be of therapeutic value prior to total thyroidectomy. Therefore this procedure, which has been effective in increasing the avidity of these metastases for I^{131} (1, 2, 3), is usually carried out prior to radioactive iodine treatment. In increasing the uptake of I^{131} by means of surgical total thyroidectomy, growth of metastases inevitably follows (Table 2). To obviate this sudden growth stimulus, it was thought that I^{131} therapy might be advisable in patients who show an uptake of I^{131} in the metastases after partial thyroidectomy. In the 9 patients in this series in whom a partial thyroidectomy had been performed because of a thyroid tumor, a slight but definite uptake of I^{131} was noted in the metastases of 5 (Cases 7, 8, 10, 16 and 17) prior to total thyroidectomy. In Patient M.D. (Case 17) radioactive iodine therapy was initiated without performing a surgical total thyroidectomy, but death from pulmonary insufficiency prevented a long-term ap-

praisal of this program. Frantz *et al.* (14) in 1944 reported observations on a patient with metastatic thyroid carcinoma who had had a partial thyroidectomy for removal of a thyroid tumor seven years prior to their studies. Since one metastasis was quite avid for I^{130}, they administered 10 mc. of the isotope. Twenty-four days later they administered a tracer dose of I^{130} and noted that the uptake of the isotope in this metastasis had been markedly depressed. Freedberg *et al.* (15) in 1950 reported observations on the successful treatment with I^{131} of a thyroid metastasis in the lung of a patient who had only a partial thyroidectomy. It is of interest that twenty days following the I^{131} treatment, the metastasis contained more of the therapeutic dose of I^{131} than did the residual thyroid. Treatment of metastatic thyroid carcinoma with I^{131} in patients who have had only a partial thyroidectomy and whose metastases concentrate I^{131} warrants further trial. Although this program will result eventually in a radiation total thyroidectomy, the gradual increase in TSH stimulation to the metastases resulting from the gradual destruction of the thyroid may be of more value in the therapeutic program than the sudden increase in TSH activity with its growth-stimulating effect, induced by surgical total thyroidectomy.

The administration of antithyroid drugs is another method of value for increasing the uptake of I^{131} in metastases of thyroid carcinoma (Table 2). The increase in the uptake of I^{131} is often associated with growth of the metastases, resulting indirectly from TSH stimulation. This factor may outweigh the benefit to be derived from increasing the avidity of the tumor for I^{131}. It is also conceivable that the antithyroid drugs may have an adverse effect directly on the tumor, as Suzuki (16) noted that methylthiouracil disturbs the normal RNA/DNA ratio in the thyroid of the hypophysectomized rabbit. Since the effect of antithyroid drugs in increasing the I^{131} uptake may be diminished once radioactive iodine therapy has been given, repeated attempts to stimulate the metastases may be unwise.

The response to radioactive iodine treatment has been gratifying in a limited number of patients with metastatic thyroid carcinoma. Pulmonary lesions regress and bone pain is usually relieved within a week after the administration of I^{131}. The relief of pain has been impressive, even in patients with a slight uptake of the isotope (less than 20 per cent of the administered dose) in the metastases. However, the functional and histologic variations in metastases in the same patient can be the cause of disappointing results.

Functional and structural variations in metastases

Concerning the functional variations, it appears that certain areas of a single metastasis are more avid for iodine than others. This concept is

based on three observations: 1) The marked difference in the uptake of I^{131} and in the radiation effect noted in a single metastasis following a therapeutic dose of I^{131} (Figs. 3 and 4). Normal and hyperplastic thyroid tissues also have a patchy concentration of I^{131} (17). However, recently Fitzgerald (18) demonstrated by means of quantitative microdensitometry that there is a greater variation in follicular I^{131} concentration in thyroid carcinoma, especially follicular carcinoma, than in normal thyroid tissue. 2) The change in the serum PBI level following therapeutic doses of I^{131} (Fig. 5). On the basis of such data, it is assumed that only portions of the metastases become sufficiently irradiated at any one time to induce the rise in serum PBI concentration which probably is an indication of tissue damage (7). 3) The lowering of the serum PBI level by the oral administration of thiouracil (Fig. 6) in twenty-five days and the histologic evidence for the focal concentration of colloid in the metastasis suggest that the administration of thiouracil depletes a small store of thyroid hormone from only a portion of the large tumor mass.

This hypothesis that certain areas of a single metastasis are more avid for iodine than others may explain the limited vulnerability of a metastasis to a single dose of I^{131}. Following the damage or destruction of these areas, other portions of the metastasis may then be stimulated to have an increased avidity for I^{131}. Hence, it would appear that the cancericidal effect of any single dose of I^{131} is limited, and that further tumor destruction requires additional radioactive iodine therapy.

In spite of a favorable response of some metastases to I^{131} therapy, the physician is often defeated in his attempt to eradicate all disease by the poor response of other metastases in the same patient. This is related to the variability in the histologic differentiation and function of these metastases. Simpson (19) in 1926 demonstrated this histologic variation. Recently Rawson *et al.* (20) stated that functional variations in different metastases in the same patient are limiting factors in the treatment with I^{131}. One metastasis may concentrate and retain I^{131}; others may concentrate very little; still others may concentrate the isotope and rapidly discharge it (3). In spite of this rapid turnover of the I^{131} by certain metastases, the evidence suggests that organic binding of the concentrated iodide occurs. Keston *et al.* (21) in 1942 showed that the accumulated I^{130} in the metastasis of a 39-year-old woman could not be discharged by the administration of stable iodine (I^{127}). Seidlin *et al.* (22) demonstrated that a rib metastasis was forming thyroxine; by means of chemical analysis of the iodinated substance in the tumor they found a thyroxine fraction of 17.2 per cent of the total iodine (0.192 mg. per gram of dry tissue). Robbins *et al.* (23, 24) reported a new iodinated compound in the serum of patients with metastatic thyroid carcinoma following thera-

peutic doses of I^{131}. It is precipitable with serum proteins, is not extractable in butanol, is 35 per cent extractable in acid-butanol, does not move from the origin in filter paper chromatography, migrates with serum albumin during zone electrophoresis, and yields only monoiodotyrosine on alkaline hydrolysis. This compound could be thyroglobulin which is only partially iodinated[4] and may be indirect evidence that incompletely iodinated thyroglobulin is present in some metastases. Thus it appears that thyroid metastases exhibit variations in their ability to concentrate, bind and release I^{131}. That their response to I^{131} treatment is variable is not surprising.

Complications of I^{131} therapy

Complications of I^{131} treatment have been relatively insignificant, except for changes in the hematopoietic system (Table 3). During a program of desiccated thyroid therapy and no further I^{131} (Fig. 9), the hematopoietic abnormalities in Case 5 disappeared except for the low white blood cell count and the erythroid hyperplasia in the bone marrow. Trunnell *et al.* (25) noted erythroid hyperplasia in the bone marrow of several patients following I^{131} therapy and found that the myeloid erythroid ratio became normal in about six months.

The most serious changes reported to follow large doses of I^{131} have been in the hematopoietic system (25–28). A transient drop in the lymphocyte count has been frequently observed, but has been of no obvious import. Rall *et al.* (29) noted a decreased incorporation of radioactive iron into the red blood cells following I^{131} treatment. This could explain the failure of oral ferrous sulfate to raise a low level of hemoglobin in the blood of several of our patients during periods of from four to six months after large doses of I^{131}.

Trunnell *et al.* (25) reported that fatal pancytopenia developed in a 38-year-old woman one month after the administration of a single dose of 250 mc. of I^{131}. Prior to this treatment, she had received external radiation and a total dose of 277 mc. of I^{131}. Blom *et al.* (27) reported the development of leukemia in a patient who had received 3,900 r of external radiation in 1948 and a total dose of 261 mc. of I^{131} from 1951 to 1952. Twelve of our patients received both external radiation in doses of from 1,500 r to 5,200 r and I^{131} in total doses of 62 to 1,300 mc. In only Patient M.S. (Case 5) did changes develop in the hematopoietic system (Fig. 9). In this patient, hematologic abnormalities were present prior to the administration of the second form of radiation. Seidlin *et al.* (28) reported the occurrence of myeloid leukemia in 2 of 16 patients treated with total doses of 195 to 2,290 mc. of I^{131} over periods of from six months to nine years. In the

[4] Suggested to the authors by Dr. J. Lerman.

first patient, a 62-year-old man who had an anaplastic carcinoma of the thyroid with metastases to lymph nodes, skull, spine and lungs, leukemia developed four years after he received a total dose of 1,455 mc. of I^{131}. In the second patient, a 66-year-old female, leukemia developed five years after the administration of a total dose of 1,600 mc. of I^{131}. The conclusion of these authors was, "we do not claim that a causal relationship between radioiodine therapy and leukemia is definitely established by these results. However, the occurrence of these two cases in a series of sixteen patients makes the presumptive evidence strong for the correlation."

Inhibition of pituitary activity

The restricted usefulness of I^{131} in the treatment of patients with metastatic thyroid carcinoma led to the administration of desiccated thyroid and testosterone in an attempt to inhibit pituitary activity and thus inhibit growth of the metastases. Frantz *et al.* (26) in 1950 stated that the administration of desiccated thyroid may have been a factor in the apparent arrest of the growth of metastases from thyroid carcinoma in 2 patients. However, it appears from their data that the arrested growth could have been due primarily to the continued administration of I^{131}. Balme (30) reported that pulmonary metastases from a thyroid carcinoma were successfully treated by the administration of thyroxine but, from the data, it is difficult to conclude that the treatment was successful or that any success achieved was attributable to the administration of thyroxine and not to I^{131} which the patient had received. The effects of desiccated thyroid therapy in 6 euthyroid patients in our series with metastastic thyroid carcinoma suggest that this program has been of value only in controlling the growth of metastases which had been sensitive to I^{131} treatment, and therefore may be used as an adjunct in the overall therapy.

In 1951, Lemon *et al.* (31) reported observations on the administration of testosterone to a woman, 53 years of age, with supposed metastatic thyroid cancer. A dramatic recalcification of the bony metastases was noted. From our experience, the presence of osteoblastic metastases in this patient raises some doubt about the diagnosis of thyroid carcinoma. The administration of testosterone propionate for two to twelve months in 4 of our patients had no obvious effect on either the function or growth of their metastases.

SUMMARY

Total thyroidectomy and the administration of the antithyroid drugs are effective in inducing or increasing an uptake of I^{131} in the metastases of patients with thyroid carcinoma. However, this effect may be outweighed by the concomitant growth stimulus imparted to the metastases.

Functional and histologic variations in metastases of the same patient

may defeat the attempt of the physician to treat thyroid carcinoma successfully with I^{131}.

General bodily complications resulting from the administration of total doses of from 70 to 2,000 mc. of I^{131} over periods of one to eight years have not as yet been limiting factors in treatment with this isotope.

Desiccated thyroid, but not testosterone, appears to be of value in controlling the growth of thyroid metastases which have been sensitive to I^{131} treatment.

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RELATIONSHIP BETWEEN DIURNAL VARIATIONS IN URINARY VOLUME AND THE EXCRETION OF ANTIDIURETIC SUBSTANCE*

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IN THE normal individual there is a diurnal cycle of excretion of water and solids which is maximal during the daytime hours of normal activity (1–4). However, in several disease states associated with water and sodium retention, including cardiac failure and hepatic cirrhosis with ascites, this cycle of water and sodium excretion may be reversed, and maximal output is during the night (5). It was postulated that if variations in tubular activity regulate this fluctuation in water and sodium excretion, it might be possible to demonstrate appropriate variations in a controlling metabolite or hormone. Thus, during periods of antidiuresis there might be a large amount of antidiuretic substance in the urine, whereas during periods of diuresis the amount of antidiuretic substance might be relatively small. A check upon this relationship could be obtained by observing individuals with both normal and abnormal patterns of urinary excretion. It was hoped that this approach might help clarify the mechanisms of normal and abnormal diurnal cycles of urine formation, and perhaps also help explain the increase in antidiuretic substance (ADS) which has been reported in the urine of patients with water and salt retention (6–11).

METHODS AND MATERIALS

Previous studies (12) indicated that large differences in urinary volume could be obtained if two eight-hour specimens were collected, one from 10 P.M. to 6 A.M., the other from 6 A.M. to 2 P.M. In normal individuals the daytime volume is the greater, whereas in most patients with heart failure or cirrhosis with ascites, the reverse is true. Since it was proposed to determine what factors caused differences in urinary volume, the urines were purposely selected so that the larger volume was at least one and one-half times that of the smaller. Urine specimens were all obtained from adult men, and included 9 normal subjects, 6 patients with congestive cardiac failure and 5 patients with cirrhosis of the liver and ascites.

The urinary ADS was prepared for assay by a modification of the method of Noble,

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Rinderknecht and Williams (13), which utilized the fact that ADS can be readily adsorbed and eluted from a precipitate of zinc ferrocyanide. This technic was used in order to avoid losses of ADS which are introduced by dialysis methods (14–18). Urines to be assayed were refrigerated during collection, and the pH adjusted to 5.0 with glacial acetic acid as soon as they were received in the laboratory. The entire sample was used. In our hands the final solution as described by Noble *et al.* resulted in a high mortality when injected into rats. For this reason the ADS was further purified by ether precipitation (19), and the resultant material was easily soluble in 10 ml. of isotonic sodium chloride solution. All extracts were dissolved in the same volume of saline solution so that direct comparisons of the absolute amount of ADS per unit of time could be made. The pH of the extract was approximately the same as that of beta-hypophamine (Pitressin) in isotonic sodium chloride solution, which was used as a standard. In two years of experience with this extract, there were no deaths of rats following its injection.

The bioassay was the Ham and Landis (15) modification of the Burn (20) method. Male rats of the Long-Evans strain in the weight range of 150 to 250 grams were used. Each animal was injected with 1.0 ml. of urine extract. A complete assay usually involved: 1) two caged groups of 3 rats each, for injection with a control solution of isotonic sodium chloride, 2) two caged groups for injection of each of the two unknown solutions, and 3) two caged groups for injection of a standard solution of Pitressin in isotonic sodium chloride. The cumulative urinary excretion by each cage of rats was plotted against time on rectangular coordinate paper for a period of 180 minutes, and the area under the curve was measured by a planimeter. The numerical value for the area was the assay result. The assay result was then subtracted from the area plotted for the sodium chloride controls, in order to obtain an assay value.

RESULTS

The validity of the assay method was tested, using known solutions of Pitressin at dose levels of 1.0, 2.5 and 5.0 milliunits. The data are presented in Table 1. The response showed a log-dose relationship which is defined by the equation $y = 19.8 + 47.2 \log x$, where x is the dose in milliunits of Pitressin, and y is the difference between the control and assay areas in square centimeters.

A series of 5 recoveries was performed, to determine whether the method of urine extraction actually concentrated a substance similar to, if not identical with beta-hypophamine. Five milliunits of commercial Pitressin were added to aliquots of normal urine. The results of these recoveries are shown in Table 2. The mean recovery of the added Pitressin was 109 per cent, which is within the range of error of the method, although more that was anticipated. Noble *et al.* (13) have also reported recovery values of over 100 per cent, which they attributed to the simultaneous extraction of augmentor substances; however, these were effective only on subcutaneous, and not on intraperitoneal injection.

As a result of the apparent validity of the assay and recovery methods, the amount of ADS in the urine was read directly from the dose-response curve and expressed in terms of milliunits of Pitressin. It is recognized that

TABLE 1. STANDARD ASSAYS OF COMMERCIAL PITRESSIN

Dose in milliunits	No. of assays	Av. area of assay (sq. cm.)	Av. assay value (sq. cm.)	Std. Dev.	Calc. assay value (sq. cm.)*
0.0	15	90.4			
1.0	10	67.6	22.8	14.7	19.8
2.5	4	52.3	38.1	15.4	38.5
5.0	12	37.7	52.7	15.8	52.8
5.0†	53		51.0	12.6	

* Calculated by method of least squares.
† Mean value for all 5.0-mu. Pitressin assays.

two assays of each urine are insufficient to yield precise absolute values by this method.

The urinary volumes, the amounts of ADS recovered, and the day-to-night ratios are presented in Table 3. The urines of 9 normal subjects were assayed in 10 paired urine collections. Antidiuretic substance was found in all, and in only 1 was the amount less than 10 milliunits in sixteen hours. In 7 the larger amount of ADS was found in the urine of larger volume, and in 3 the larger amount of ADS was found in the urine of smaller volume. Assays were also performed on the urines of 5 patients with cirrhosis of the liver with ascites, and 6 patients with cardiac failure. In these subjects the night urine specimen was the larger. All but 1 demonstrated more than 10 milliunits of ADS in the sixteen-hour urine collection. In 8 the larger urine contained the greater amount of ADS, and in 3 the larger amount was found in the smaller urine. Thus, in 21 pairs of determinations, the greater ADS correlated with the larger urinary volume in 15, and with the smaller urinary volume in 6. Thus the experimental premise that a greater amount

TABLE 2. ASSAYS OF COMMERCIAL PITRESSIN RECOVERED FROM URINE

Substance assayed	No. of patients	No. of assays	Av. area of assays (sq. cm.)	Av. assay value (sq. cm.)	Std. Dev.	Assay values in milli-units
Sodium chloride solution (control)		8	92.1			
Urine	4	8	78.4	13.7	8.9	.75
Pitressin (5.0 milliunits)		9	39.5	52.6	17.1	5.0
Urine+Pitressin (5.0 milliunits)	5	10	34.9	57.2	17.1	6.2

TABLE 3. COMPARISON OF URINARY VOLUMES AND ADS VALUES

Patient	Urinary volume				Antidiuretic substance			
	Day (ml.)	Night (ml.)	Total (16 hrs.)	Ratio day/night	Day (m.u.)	Night (m.u.)	Total (16 hrs.)	Ratio day/night
Normal subjects								
R.G.	365	115	480	3.16	24.3	15.8	40.1	1.54
R.G.	380	250	630	1.52	65.0	20.8	85.8	3.02
G.S.	690	225	915	3.07	26.2	23.0	44.2	1.14
R.A.	510	270	780	1.89	14.6	27.0	41.6	0.54
C.B.	1200	400	1600	3.00	10.8	12.8	23.6	0.84
W.W.	750	275	1025	2.73	23.0	20.7	43.7	1.11
M.S.	340	155	495	2.19	63.0	21.9	84.9	2.88
S.L.	465	300	765	1.55	73.5	28.1	101.6	2.61
R.R.	1620	620	2240	2.61	22.2	21.8	44.0	1.02
E.H.	600	370	970	1.62	0.0	4.7	4.7	0.00
Cirrhosis with ascites								
C.N.	580	1400	1980	0.41	27.5	171.3	198.8	0.16
G.D.	210	660	870	0.32	33.4	96.0	129.4	0.34
R.H.F.	305	660	965	0.46	9.1	21.2	30.3	0.43
W.J.H.	128	256	384	0.50	8.1	4.2	12.3	1.93
R.E.R.	305	505	810	0.60	10.0	11.2	21.2	0.90
Congestive cardiac failure								
W.M.	136	256	392	0.53	34.0	15.2	49.2	2.23
W.H.N.	450	1210	1660	0.37	6.3	13.2	19.5	0.48
F.O.B.	148	910	1058	0.16	6.8	0.0	6.8	infinite
C.P.D.	285	860	1145	0.33	9.7	10.7	20.4	0.91
G.P.	240	510	750	0.47	7.1	15.3	22.4	0.46
A.J.T.	185	570	755	0.32	4.4	7.5	11.9	0.59

of ADS would be found in the urine of smaller volume was not confirmed. Examination of the results suggests that the opposite may actually be the case, and that the larger volume and the larger ADS assay may be associated more significantly than by random distribution alone.

It should be noted that not only was a significant amount of ADS recovered from the urine of normal individuals, but that, in general, more was recovered from the normal group than from patients with cardiac failure and edema. In 3 of the 5 urines from patients with cirrhosis the ADS titers were low or within normal range, but in the other 2 patients they

were so high as to make the average considerably higher than the normal. The total sixteen-hour ADS excretion tended to demonstrate a reciprocal relationship to the amount of urine excreted during that period, when compared with that of the other patients, even though this correlation did not hold for the shorter periods in the same patient. This should rule out the possibility that the ADS which was recovered was actually an artifact related to the original urinary volume. Two patients with cirrhosis were obvious exceptions to this observation, and 1 in particular demonstrated an unusually high assay with a very high urinary flow.

DISCUSSION

If ADS represents pituitary antidiuretic activity and is the factor causing antidiuresis, the amount reaching the tubules per unit of time must be greater in absolute amount than during a period of diuresis. If the urinary concentration of ADS reflects the amount of ADS acting upon the tubules, then the amount in the urine also must be absolutely greater during antidiuresis, not merely greater in terms of urinary concentration. This does not appear to be the case. It may be that the posterior pituitary hormone ordinarily sets the general rate of water loss in association with other mechanisms which regulate the diurnal rates of water loss. The response of the posterior pituitary to a sudden osmotic stress may be an emergency function which is infrequent, but one which could conceivably obscure the basic pattern as determined by the methods of this study.

Urinary assay may not be a valid measure of the amount of effective ADS, for reasons other than those of analytical technic. For example, since voiding is less frequent during periods of antidiuresis, there is more time for ADS inactivation within the bladder. Also, Pitressin is known to be absorbed through the nasal and vaginal mucous membranes, and it may be reabsorbed through the vesical mucous membrane as well. The combination of prolonged stasis and a smaller urinary volume producing a greater ratio of bladder area to urinary volume may result in increased losses by this route. It is apparent that the answers to these questions may lie in appropriate assays of ADS levels in blood.

Several observers have reported the absence of ADS in the urine of normal individuals as compared to its presence in the urine of patients whom they happened to be studying. In most of these procedures the urine for assay was prepared by dialysis (6, 7, 8, 10). It has been amply demonstrated that a large proportion of the antidiuretic activity is lost after dialysis (14–18). This is compatible with the recent demonstration by du Vigneaud and his associates that Pitressin is a polypeptide with a molecular weight of approximately 1,000 (21). Perhaps the ADS which was recovered from normal urine was of this type, and was retained by the adsorption

technic used, whereas in disease states the effective portion of the ADS is part of a larger protein molecule which does not diffuse across the dialysis membrane. Ham and Landis (15) found that commercial Pitressin was dialyzable, but that the urinary ADS which they measured was not. However, this explanation is weakened by the original work of Noble *et al.* (13); they found no ADS in 1 normal urine. Ellis and Grollman (22), using an adsorption technic of their own, found only trace amounts of ADS in the urines of 9 normal individuals. However, Stein, Jinks and Mirsky (17), using the Grollman adsorption technic, recently reported an average of 15 milliunits of Pitressin equivalent for 10 normal urines, with a range from 6.3 to 24.7 milliunits per day. This compares with our average of 51.4 milliunits, with a range of 4.7 to 101.6 milliunits per sixteen hours. In addition, the recent observations of Stein, Schwartz and Mirsky (23), and of Perry and Fyles (24) that there is ADS activity in normal blood serum, and that there may not be abnormal amounts of ADS in the serum of patients with cirrhosis and cardiac disease, challenge the significance of the reports concerning the increased amounts of ADS in the urine of such patients.

CONCLUSIONS

1. The urines of 9 normal subjects, 6 patients with cardiac failure, and 5 patients with hepatic cirrhosis and ascites were assayed for antidiuretic substances by a modification of the extraction method of Noble *et al.* and the bioassay technic of Burn.

2. Contrary to results of previously published studies, the normal subjects were found to excrete as much or more ADS as patients with cardiac and hepatic disease. It is possible that the modified recovery method retained ADS which is ordinarily lost in dialysis.

3. Comparison of urines obtained during periods of diuresis (morning collections in normal subjects; night collections in patients) with urines obtained during periods of antidiuresis revealed that in 15 paired specimens the greatest recovery of ADS coincided with the larger urinary volume, and in 6 the greatest recovery of ADS coincided with the smaller urinary volume.

4. These observations cannot be reconciled with the concept that the rate of urinary flow under these circumstances is inversely related to ADS activity, or that ADS is the factor which regulates the diurnal rhythm of urinary flow.

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STRUMA LYMPHOMATOSA: PRIMARY THYROID FAILURE WITH COMPENSATORY THYROID ENLARGEMENT*

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INTRODUCTION

SINCE struma lymphomatosa was first described by Hashimoto (1) in 1912, there has been increasing opinion that this disease is not a true thyroiditis (2–9). The purpose of this paper is to present clinical, physiologic, biochemical, pathologic and therapeutic observations to substantiate the belief that struma lymphomatosa is idiopathic primary failure of the thyroid cells themselves with compensatory hyperplasia and secondary lymphocytic infiltration and fibrosis.

REVIEW OF THE LITERATURE

The clinical features of the disease, physical characteristics of the goiter, basal metabolic rates and serum cholesterol values have been described previously by other investigators (3, 4, 8, 10, 11), as have the pathologic features (1, 3, 6, 7, 8, 10, 12).

Radioactive iodine[1] uptakes have been recorded as ranging from low to high (10, 11, 13–15), although almost all of those reported were normal. The TSH test has been established as a valuable aid in the diagnosis of thyroid failure without goiter (16–22). Levy and associates (23) noted abnormal results with the TSH test in children whom they believed to have congenitally defective thyroids and in 1 adult; all of these patients had decompensated thyroid failure with goiter, but no pathologic studies were made.

Serum lipoprotein studies on patients with thyroid failure without goiter

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[1] The radioactive iodine used in this investigation was supplied by the Abbott Laboratories on authorization from the Isotopes Division, U. S. Atomic Energy Commission.

revealed an increase in most of the fractions, but particularly of the beta lipoprotein fraction (24, 25), Tiselius protein studies on patients with thyroid failure without goiter showed decreased albumin and alpha-globulin and increased beta- and gamma-globulins in most patients (26). The occurrence of abnormal colloidal gold curves in 2 patients with struma lymphomatosa was reported by Cooke and Wilder (27).

Statland and associates (10) noted that serum total protein-bound iodine (TPBI) values were 0.9, 2.5 and 4.4 μg. per 100 ml. in 3 adult patients with struma lymphomatosa. Gribetz and associates (15) in a study of 6 children with struma lymphomatosa, noted high TPBI values of 8.8, 9.6 and 9.3 μg. per 100 ml. in 3 patients, the first 2 of whom had butanol-extractable protein-bound iodine (BEPBI) values of 3.7 and 4.2 μg. per 100 ml., respectively. These workers (15) suggested that the disparity between the TPBI and BEPBI values was due to formation of a metabolically inactive hormone.

The use of desiccated thyroid to decrease the size of the goiter of struma lymphomatosa has been advocated by previous investigators (7, 10, 11, 15).

MATERIALS AND METHODS

Careful clinical studies were made on 36 patients. In each case, the diagnosis of struma lymphomatosa had been proved by needle biopsy. Duplicate tests of the basal metabolic rate were made and the results averaged. Serum cholesterol determinations were made in duplicate, according to the method of Abel and associates (28), and the results averaged.

Twenty-four-hour radioactive iodine uptakes were measured by direct count over the thyroid gland with a scintillation counter after the oral administration of 20 to 50 μc. of I^{131}, either as a capsule or as a liquid. None of these patients had a history of having received iodine in any form (including desiccated thyroid) in a recent period preceding these studies. Thyrotropic hormone tests were performed, using 4 U.S.P. units of thyrotropin.[2] The procedure was a modification of that described by Jefferies and associates (20). Doses of approximately 20 and 40 μc. of I^{131}, respectively, were given usually on successive days in capsule or liquid form at least three hours after a meal; measurements were made usually at the end of six instead of three hours. Patient 2 had a 24-hour TSH test and Patients 7 and 21 had three-hour tests. At present we prefer the six-hour TSH test over the fasting three-hour TSH test because of the possible error due to differences in rates of intestinal absorption of I^{131}, although the latter test may be just as satisfactory.

Serum lipoprotein determinations were performed, using the method of Lewis, Green and Page (29). Fractional electrophoretic studies of the serum proteins were made, using Longworth's modification of the Tiselius procedure (30). Colloidal gold tests were carried out on the blood serum, using the method provided to us by Cooke (31) and modified by Alfred Reich in our serologic laboratory. Each time that a group of sera suspected of being abnormal was tested, a normal control also was tested. A new method

[2] Armour Laboratories, Chicago, Ill.

(devised by Alfred Reich) of adjusting the pH of the commercial Lange colloidal gold solution through the use of known normal and abnormal sera yielded more consistently accurate results than had been observed previously when commercial Lange colloidal gold solutions were used. Thymol turbidity and zinc sulfate turbidity tests were also performed on some patients.

Serum total PBI and butanol-extractable PBI values were determined in duplicate or triplicate by the method described by Barker (32). In the case of BEPBI, the extraction was carried out by the method described by Man and associates (33). The blood was drawn before TSH was given. All of these determinations were performed by the same experienced technician under direct supervision of one of us (H.B.)

Needle biopsy specimens of the goiter were obtained in the 36 patients, using the method described by Crile and Vickery (34); the microscopic sections were stained with hematoxylin-eosin.

RESULTS

Clinical features

The clinical features of the 25 patients with struma lymphomatosa who had complete studies are presented in Table 1. The clinical features of the 11 other patients with struma lymphomatosa and less complete studies will not be presented in detail but are summarized in the following discussion.

The 36 patients were aged from 12 to 59 years, the majority being between 30 and 50 years. There were 33 women and 3 men. Three women noted enlargement of the thyroid gland within one month, and 3 within three months after termination of pregnancy.

The duration of the goiter in most of these patients was less than two years and often was only a few weeks or months. However, there were some in whom the duration was longer, the longest being eight years without development of clinical evidence of thyroid failure. In many of these patients there was a history of sudden rapid enlargement, but some did not know they had a goiter until it was discovered on routine physical examination. In contrast, most patients with nontoxic adenomatous goiter had known of its presence for more than two years.

The sizes of the goiters varied from slightly larger than the normal 25-Gm. gland to an estimated 150 Gm. All the goiters were diffusely enlarged; the surfaces of most were smooth and those of others were lobulated. In some, one lobe was larger than the other. No frankly nodular goiter was observed in this series and no enlargement of one lobe only, such as occurs in some patients with nontoxic adenomatous goiter. In a few instances the pyramidal lobe was enlarged enough to give the impression that a nodule was present, but actually this was a part of the diffuse enlargement.

The consistency of the goiter was usually firm or very firm; in a few patients it was hard, and in 1 it was soft.

Despite the presence of these characteristic physical features of the goiter of struma lymphomatosa, it was often difficult for the experienced

TABLE 1. CLINICAL, THERAPEUTIC, PATHOLOGIC AND THYROID FUNCTION STUDIES OF 25 PATIENTS WITH STRUMA LYMPHOMATOSA

Patient			Estim'd size of goiter		Duration of goiter	Clinical thyroid failure	B.M.R. (%)		Serum TPBI** (μg./100 ml.)		Serum BEPBI** (μg./100 ml.)		TSH test (I^{131} uptake; %)		Daily dose of thyroid	Pathologic pattern (type)
No.	Age (yrs.)	Sex	Pre-* (Gm.)	Post-* (Gm.)			Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-		
1	42	F	50 Firm	35	8 yrs.; rapid enl. 2 mo.	Absent	−13	− 9		4.0				6, 12	3 gr. 3 mo.	Hashimoto
2	23	F	65 Firm RL Soft LL	35	4 yrs.; grad. enl. 1 yr.	Absent	+ 8	+22	7.5	4.6	4.0	4.3	50, 58		2 gr. 2 mo.	Hyperplastic
3	25	F	50 Firm		1 mo.	Absent	+ 2		3.8		2.0				3 gr.	Hyperplastic
4	51	F	50 Firm	30	4 yrs.; rapid enl. 6 mo.	Absent	+ 4	− 2	4.2		2.2		36, 32		1 gr. 2 mo.	Intermediate
5	37	F	85 Firm	85	6 mo.	Absent	−11	−10	3.8		3.5		34, 41		2 gr. 2 mo. (convulsive disorder)	Hashimoto
6	34	F	85 Firm	50	1½ yrs.	Absent	−15		4.4		3.8		31, 34		3 gr.	Hashimoto
7	25	F	50 Firm		1 mo.	Absent	−19		3.7		2.7		24, 20		3 gr.	Hyperplastic
8	40	F	50 Firm		8 yrs.	Absent	−21		5.3		4.0		42, 37		3 gr.	Hashimoto
9	59	F	75 Firm R>L		2 mo.	Absent	+ 5	−4	6.6	5.7	3.9	5.5	40, 46		3 gr. 2 mo.	Hashimoto
10	40	F	150 Firm	50	4 yrs.; rapid enl. 1 yr.	Absent	−15	+1						6, 34 10 units TSH	3 gr. 9 mo.	Hashimoto
11	47	F	60 Firm	30	1½ yrs.	Absent	−8	+11						8, 12	3 gr. 4 mo.	Hashimoto
12	58	F	60 Recur. 2 yr. post-op. Firm	30	3 yrs.	Absent	−18	+11	6.7		4.5	5.6	29, 21		3 gr. 2 mo.	Hashimoto
13	32	F	75 Firm	40	3 mo.	Absent	−11	−6						10, 6	3 gr. 2 mo.	Hashimoto

Patient			Estim'd. size of goiter		Duration of goiter	Clinical thyroid failure	B.M.R. (%)		Serum TPBI** (μg./100 ml.)		Serum BEPBI** (μg./100 ml.)		TSH test (I^{131} uptake; %)		Daily dose of thyroid	Pathologic pattern (type)
No.	Age (yrs.)	Sex	Pre-* (Gm.)	Post-* (Gm.)			Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-		
14	54	F	60 Hard	50	Unknown	Present; mild	−17	+32	4.1	8.8		7.0	24, 30	13, 9	3 gr. 2 mo.	Hashimoto
15	41	F	60 Firm		4 mo.	Present; mild	−21		5.0				58, 47		3 gr.	Hyperplastic
16	38	F	50 Hard		Unknown	Present; mild	−32				2.3		51, 44		1 gr. (convulsive disorder)	Hashimoto
17	12	F	125 Firm	60	2 yrs.	Present; mod.	−25	− 4	5.1		2.5	8.2	98		0.3 mg. *l*-thyroxine	Hashimoto
18	41	M	60 Hard	50	2 yrs.	Present; mod.	−29	−14					7, 4		3 gr. 2 mo.	Hashimoto
19	30	F	100 Firm	50	1½ yrs.	Present; mild	−16	+ 4							3 gr. 4 mo.	Intermediate
20	45	F	50 Firm	30	Unknown	Absent	− 8	+17					24, 30		3 gr.	Hashimoto
21	36	F	60 Firm	40	2 yrs.	Absent	− 6	+28		6.5			24, 22		2 gr. 2 mo.	Hashimoto
22	46	F	50 Firm	40	1 yr.	Absent	+ 3	+40		10.8			57, 47		3 gr. 2 mo.	Hashimoto
23	55	F	50 Firm	40	Unknown	Present; mild	−18	− 2					61, 51		0.3 mg. *l*-thyroxine, 2 mo.	Hashimoto
24	32	F	85 Firm	50	4 mo.	Absent	−23	− 3			4.0		60, 46	6, 34 20 units TSH	0.3 mg. *l*-thyroxine, 2 mo.	Hashimoto
25	35	F	60 Firm	40	4 wks.	Absent	−15	− 9					20, 20		2 gr. 2 mo.	Hashimoto

* Pre = Before thyroid therapy. Post = After thyroid therapy.

** TPBI = Total protein-bound iodine; normal range, 3.8–7.8 μg./100 ml. BEPBI = Butanol-extractable protein-bound iodine; normal range, 3.2–7.2 μg./100 ml.

observer and particularly difficult for the inexperienced observer to differentiate this type of goiter from nontoxic adenomatous goiter without further studies. Moreover, because of these physical features, this goiter has been mistakenly termed simple or colloid goiter.

Only 10 of the 36 patients had symptoms and/or signs of thyroid failure, varying from mild to moderate. Two of these 10 patients had myxedema. In some, direct questioning was required to elicit the fact that there had been symptoms which disappeared after treatment. None of the patients had a history of hyperthyroidism.

We have arbitrarily classified the thyroid failure in these cases of struma lymphomatosa as compensated or decompensated. We have defined "compensated thyroid failure" as the state in which there are no symptoms and/or signs of thyroid failure despite laboratory evidence indicating otherwise; and "decompensated thyroid failure" as that in which symptoms and/or signs of thyroid failure are present.

Basal metabolic rates

Regardless of the presence or absence of symptoms and signs of thyroid failure, 22 of 36 patients had a basal metabolic rate (BMR) of −15 per cent or below. The BMR's of 25 of the 36 patients are recorded in Table 1. In the patients with low rates who received 2 or 3 grains of desiccated thyroid and had recheck determinations, there was a significant increase in BMR, usually greater than 20 per cent (Table 1). In some patients with rates above −15 per cent there was also a significant increase in response to thyroid (Table 1). This response to desiccated thyroid in physiologic doses is added evidence of the presence of thyroid failure which may be asymptomatic. In contrast, the basal metabolic rates of euthyroid patients with or without adenomatous goiter are not significantly elevated by such doses.

Total and butanol-extractable protein-bound iodine of serum

Determinations of serum TPBI (normal 3.8–7.8 μg. per 100 ml. in our laboratory) were made in 12 patients. Results were in the low-normal range in 9 patients and in the high-normal range in 3 (6.6, 6.7 and 7.5 μg. per 100 ml., respectively) (Table 1). Other evidence of thyroid failure (Table 1) included hyperplasia in all; low basal metabolic rates and elevated serum cholesterol levels in many; failure to respond to TSH in all; and low or low-normal BEPBI values in all. These findings seem consistent with the generally low-normal TPBI values. The presence of normal TPBI values indicates that this test alone is of little value as an aid in the diagnosis of thyroid failure in most patients with struma lymphomatosa.

BEPBI values (normal 3.2–7.2 μg. per 100 ml. (33)), which are regarded as a more accurate measure of thyroxine levels than are TPBI values (33), were low or low-normal in all of 12 patients. This is further evidence that

these patients had some degree of thyroid failure (Table 1), and also indicates that BEPBI may be a more sensitive index of mild degrees of thyroid failure with goiter than is TPBI. Nevertheless, the BEPBI test was not a definitive method of diagnosing thyroid failure in some of these patients in whom values were in the low-normal range. It seems likely, for example, that the BEPBI level may have been 6.0 μg. per 100 ml. before the onset of thyroid-cell failure and decreased to 4.0 μg. per 100 ml. after thyroid failure. The normal limitation of error of the procedure (1 μg. per 100 ml.) decreases its value as a definitive method. Unfortunately, the even greater difficulty in performing the BEPBI determination, as compared with the difficult TPBI determination, diminishes the value of this test for routine clinical use at present.

In a few patients TPBI and BEPBI determinations were made after treatment with desiccated thyroid. These values, especially for BEPBI, were usually increased; however, TPBI levels were decreased in 2 patients (Table 1).

The average disparity between TPBI and BEPBI values has been reported to be 0.6 μg. per 100 ml., with a maximal difference of 2.0 μg. per 100 ml. in normal subjects, in hypothyroidism without goiter, and in hyperthyroidism (33). The average TPBI-BEPBI disparity in a group of 17 control determinations in our laboratory (on euthyroid and hyperthyroid patients and those with struma lymphomatosa taking desiccated thyroid) was 0.73 μg. per 100 ml., with a maximum of 2.5 μg. per 100 ml. In 10 patients with struma lymphomatosa the average disparity was 1.8 μg. per 100 ml. In 7 of the 10 patients with struma lymphomatosa the TPBI-BEPBI disparity was greater than 1 μg. per 100 ml.—averaging 2.1 μg. per 100 ml. This increased disparity in patients with struma lymphomatosa could be due to the presence of a metabolically inactive iodinated protein, which may explain why TPBI values may be normal in the patients with decompensated thyroid failure. However, we cannot rule out the normal limitation of error of both of these procedures as a factor. Therefore, these results for the present should be regarded as inconclusive, *i.e.*, they neither confirm nor disprove the presence of an abnormal iodinated protein. Other more exact methods, such as chromatography, should be used to settle this question.

Thyroidal 24-hour uptake of radioactive iodine

The 24-hour I^{131} uptakes by the thyroid, and the corresponding basal metabolic rates of 26 patients are presented in Figure 1. In these patients with struma lymphomatosa the basal metabolic rate was a fairly reliable measure of thyroxine deficiency. The 24-hour I^{131} uptakes varied from 5 to 98 per cent. In many of the patients (Fig. 1) impairment of the function of I^{131} concentration paralleled the failure of thyroxine production, but in

several the changes in I^{131} concentration did not parallel the changes in thyroxine production. I^{131} uptakes may be normal or high in patients with compensated or decompensated thyroid failure, depending on the intrinsic ability of the cells to concentrate iodide and on the effect of increased stimulation by excess TSH secreted by the pituitary secondary to decreased

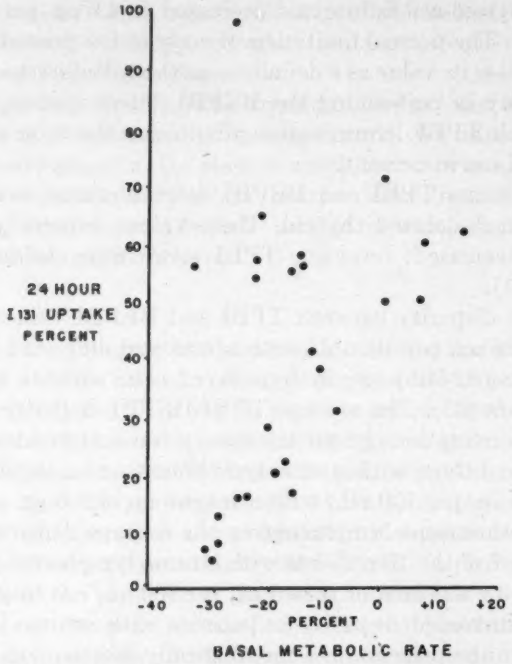

FIG. 1. Thyroidal 24-hour radioactive iodine uptakes and basal metabolic rates of 26 patients with struma lymphomatosa, illustrating that I^{131} uptakes may vary from low to high. In the presence of what is believed to be excess TSH stimulation, the higher the I^{131} uptake the more normal is the ability of the thyroid cells to concentrate iodide.

thyroxine production. Thus, in patients with struma lymphomatosa, the ability of the thyroid to concentrate I^{131} is not a good measure of deficiency of thyroxine production.

Thyroidal 24-hour I^{131} uptakes in patients with nontoxic adenomatous goiter ranged from 9 to 83 per cent, indicating that this test is of little value in differentiating the two types of goiter.

Thyroid-stimulating hormone (TSH) tests

The results of the TSH tests performed on 18 patients are presented in Table 1. In none of the patients with struma lymphomatosa did 4 U.S.P.

units of TSH induce a significant increase in the uptake of the second tracer dose of I^{131}, regardless of the initial uptake. This suggests that the thyroid cells are already undergoing maximal stimulation by increased endogenous TSH production. In contrast, 4 U.S.P. units of TSH induced a significant increase in the uptake of the second I^{131} dose (at least 50 per cent, and usually 100 per cent or more of *the original uptake*) as compared to the initial uptake in 18 patients with nontoxic adenomatous goiter (10 proved histopathologically), provided the initial I^{131} uptake was 60 per cent or below. In an occasional patient with nontoxic adenomatous goiter having an I^{131} uptake above 60 per cent, the six-hour and 24-hour TSH tests were not as suitable as a fasting three-hour test, because the difference in rates of uptake was not as well demonstrated over the longer period of time. In our experience, in the absence of hyperthyroidism, the TSH test has proved to be a simple and accurate method of differentiating these two types of goiter preoperatively.

The only other type of goiter which may offer some difficulty in differentiation is that due to subacute thyroiditis, especially if the clinical picture is atypical. In 5 such patients in whom the diagnosis was proved by needle biopsy, results of TSH tests were positive; but 6-hour I^{131} uptakes in each test were low, whereas the basal metabolic rates were normal or slightly elevated. In contrast, when the I^{131} uptake was low in patients with struma lymphomatosa, the basal metabolic rate was also low.

The TSH test was repeated in some of the patients with struma lymphomatosa while they were taking desiccated thyroid and the results are presented in Table 1. Results of the TSH test were usually positive while the patients were taking desiccated thyroid; the findings were similar to those in treated hypothyroid patients without goiter. However, if the amount of TSH was increased to 10 or 20 units, there was a significant increase in the rate of I^{131} uptake, particularly if the original I^{131} uptake was high-normal or elevated.

Serum cholesterol levels

Of the 25 patients who had serum cholesterol determinations, 9 had elevated values (greater than 250 μg. per 100 ml.) and 16 had normal values (Table 2). The administration of desiccated thyroid to the patients with elevated or high-normal values usually resulted in a significant decrease, but in patients with lower normal values there was usually no significant change. These results indicate that the serum cholesterol level is not a good measure of mild thyroxine deficiency.

Serum lipoprotein studies

Electrophoretic analyses of serum lipoproteins were carried out in 16 patients (Table 2). In most of them, no significant abnormal elevations of the

TABLE 2. SEROLOGIC STUDIES ON THE 25 PATIENTS WITH STRUMA LYMPHOMATOSA LISTED IN TABLE 1

Patient No.	Time relation to thyroid therapy	Serum cholesterol (mg./100 ml.)	Serum lipoproteins (mg./100 ml.)					Plasma proteins (Gm./100 ml.)				Colloidal gold 552000 or greater	Thymol. turb. <5	Zinc sulfate turb. <10
			−S°>70	−S (40–70)	−S(25–40)	−S(20–25)	−S(1–10)	Albumin 3.72–5.11	Globulins					
				(Sf 10–20)	β <250 mg.	α2 <20 mg.	α1		α .39–.66	β .65–1.07	γ .60–.91			
1	Pre	174	24	40	218	12	152	3.22	.46	.88	1.53	555531		
	Post	155	26	26	177	7	118	3.53	.32	.96	1.31	311000		
2	Pre	149	26	45	223	26	141	3.43	.58	.90	.91	554310		
	Post	154	24	21	212	26	208					421000		
3	Pre	138	7	7	164	14	106	3.91	.49	1.01	.89	321000	3.9	7
	Post													
4	Pre	249						3.88	.45	1.13	.83	310000	1.2	6.2
	Post	188												
5	Pre	192	16	52	259	<7	148	2.83	.41	1.04	1.49	555311		
	Post	201										554210		
6	Pre	230	<14	18	230	<14	132	3.98	.50	1.01	1.05	554100		
	Post													
7	Pre	179	12	19	235	14	188	3.72	.32	.56	.94	554210	4.4	10.2
	Post													
8	Pre	265	24	33	376	<14	235	3.80	.42	1.25	1.17	552100		
	Post													
9	Pre	274	40	80	306		118	3.83	.29	.66	.97	321000		
	Post	202										553100		
10	Pre	200	30	40	223	19	235	3.55	.24	.82	2.62			
	Post	169	28	42	282	21	177	4.56	.49	1.11	1.79	555431	10	18
11	Pre	221	45	42	259	9	259	4.30	.41	1.23	1.27	555100		
	Post	185	21	19	177	7	141	3.78	.49	.82	.82	100000		
12	Pre	414	35	56	366	21	177	4.26	.60	1.21	.65	210000		
	Post	200	56	28	223	14	175							
13	Pre	184										552100		
	Post	165						3.40	.35	.84	1.02	520000		

Patient No.	Time relation to thyroid therapy	Serum cholesterol (mg./100 ml.)	Serum lipoproteins (mg./100 ml.)					Plasma proteins (Gm./100 ml.)				Colloidal gold 552000 or greater	Thymol turb. <5	Zinc sulfate turb. <10
			−S* >70	−S(40–70)	−S(25–40)	−S(20–25)	−S(1–10)	Albumin 3.72–5.11	Globulins					
				(Sf 10–20)	β <250 mg.	α^2 <20 mg.	α1		α .39–.66	β .65–1.07	γ .60–.91			
14	Pre	310	7	19	235	19	223	3.56	.40	1.07	1.13	553100	14	13
	Post	206	33	47	194	14	141					421000		
15	Pre	236	28	48	400	14	198	3.78	.52	.93	1.21	554310	7	10.7
	Post													
16	Pre	286	9	24	177	7	166	3.78	.30	.57	1.37	554210		
	Post													
17	Pre	200						2.66	.91	1.16	1.18	554210	8.8	11
	Post	125						4.31	.46	1.01	1.11	555431		
18	Pre	170	33	42	94	9	106	3.69	.42	.93	1.21	555420	8	10
	Post	97	24	12	94	14	94	3.42	.57	.72	1.08	544100	2.5	2.4
19	Pre	174	7	12	<177	7	118	3.66	.49	.88	.86	552100		
	Post	128										431000		
20	Pre	264										555310	6.6	9.9
	Post													
21	Pre	207										553110	4.5	7.8
	Post	100										553210		
22	Pre	210										554310	4.2	8.2
	Post	96										555210		
23	Pre	514										555420	8	13
	Post	238										552200		
24	Pre	161										555555		
	Post	107						4.0	.40	.73	1.51	554310		
25	Pre	308										321000		
	Post	133												

*—S=Negative sedimentation rate at a density of 1.21.

various fractions were noted, but in Patients 8, 9 and 15 the beta-1 and alpha-1 fractions were above normal; in other patients they were in the high-normal range. These results indicate that the serum lipoprotein test is of less value than the serum cholesterol test in determining the degree of thyroid failure.

Plasma protein studies

Electrophoretic analyses of plasma proteins were performed in 20 patients (Table 2). The significant changes noted in 16 of the 20 patients were usually a decrease in the serum albumin level to low or low-normal and an elevation of the gamma globulin level. Other less significant changes were frequently a low-normal or low concentration of alpha-globulin and a high-normal or high concentration of beta-globulin. Treatment with desiccated thyroid generally induced a significant decrease in the value for gamma globulin, although usually not to normal, and an increase in the value for serum albumin. These results suggest that the changes in plasma proteins may be due to deficiency of thyroxine. This belief is further supported by the observation that the average value of the gamma-globulin fraction in 8 patients with Graves' disease was 0.68 Gm. per 100 ml., which is essentially the reverse of the value observed in struma lymphomatosa. Also, the elevation of the gamma globulin level in some patients with primary thyroid failure without goiter is further evidence that the change may be due to thyroxine deficiency. The failure of the gamma-globulin concentration to return entirely to normal in most patients after treatment with what appeared to be adequate daily doses of desiccated thyroid (3 gr.) or sodium levo-thyroxine (0.3 mg.) may be due to the short duration of the treatment. Patient 11, whose gamma-globulin level returned to normal, received desiccated thyroid for four months instead of the usual two months. However, it is possible that the dose of desiccated thyroid prescribed may have been insufficient.

Serum colloidal-gold test

Since changes in the fractions of plasma proteins occur in patients with struma lymphomatosa, it would be of practical benefit if a more simple method of measuring these changes could be devised. The serum colloidal-gold test was used to measure these changes. The precipitation of colloidal gold in solution is believed to be due to elevation of the gamma-globulin concentration, a reaction which is aided or inhibited by a decrease or increase in albumin concentration (35). The reaction was regarded as abnormal if there was complete precipitation—55—of the colloidal gold in the first two glass tubes and significant precipitation in the third tube of a six-tube test. The details of experience with the test will be reported by Skirpan and associates (36).

Results of the serum colloidal-gold test were abnormal in 20 of 25 patients (Table 2). The corresponding Tiselius protein patterns obtained in sera from 16 of these 20 patients were also abnormal. Four patients with negative colloidal-gold findings had an essentially normal plasma protein pattern. Of 11 patients with primary thyroid failure without goiter of either endogenous or exogenous origin, 6 had positive results with the colloidal-gold test. One of these patients with a colloidal-gold curve of 555421 had a serum albumin level of 3.54 Gm. and gamma-globulin level of 1.52 Gm. per 100 ml. Another patient with a colloidal-gold curve of 310000 had a serum albumin level of 3.90 Gm. and a gamma-globulin level of 0.74 Gm. per 100 ml. The serum cholesterol values in 10 of the 11 patients were above 300 mg. per 100 ml. This suggests that the serum cholesterol level is a better measure of marked thyroxine deficiency (demonstrated clinically in these patients) than is the serum colloidal-gold test. In contrast, the reverse seems to be true in mild thyroxine deficiency, such as commonly is seen in struma lymphomatosa.

Treatment with desiccated thyroid did not reverse the serum colloidal-gold curve to normal in most of our patients during the short period they were observed; neither was the Tiselius pattern restored completely to normal (Table 2).

The value of the serum colloidal-gold test is limited by its nonspecificity, *i.e.*, it may yield positive results in any disease in which there is an increased gamma-globulin level with usually a decreased albumin level. Despite this limitation the serum colloidal-gold test in our laboratory has been a simple inexpensive, qualitative, sensitive diagnostic aid in most patients with struma lymphomatosa, when the results have been correlated with other clinical and laboratory findings. Further evaluation of this test is required before its true worth can be determined.

Serum colloidal-gold tests were performed on 40 patients with nontoxic adenomatous goiter. Results were negative in 36 and positive in 4. The findings were also negative in 8 patients with Graves' disease, and in 100 normal control patients.

Thymol turbidity, zinc sulfate turbidity, and cephalin-cholesterol flocculation tests

These tests were performed on some of the patients (Table 2). Results of the cephalin-cholesterol flocculation test were negative and are not presented. The thymol turbidity was elevated in some patients and was found to be more sensitive than zinc sulfate turbidity. However, neither method was as sensitive as the serum colloidal-gold test.

Histopathology

Morphologically, on the basis of the biopsy findings, the goiters of the 36

patients were classified generally into two groups: Hashimoto type (30 cases) and hyperplastic type (4 cases) (Table 1); in 2 biopsies the lesions were regarded as intermediate and compatible with either type.

Goiters regarded as being of the Hashimoto type were characterized by hyperplasia of the thyroid cells with diffuse oxyphilia (pink-staining eosinophilic change in the cytoplasm), and small follicles separated by an infiltrate of lymphocytes at times intermingled with plasma cells, with or without fibrosis. The oxyphilia was of varying degree; the fibrosis also varied in amount, being most evident in interlobular zones, but occasionally occurring between individual follicles. At times, large irregular nuclei were present, particularly in the more oxyphilic areas. Colloid generally was diminished, and granular, or at times dense, and usually without marginal scalloping. Histiocytes were commonly present within the lumens of follicles.

The hyperplastic variant consisted of hyperplasia of the thyroid cells with slight or no oxyphilia and follicles, usually of small size, with infoldings of the epithelial layer. There were lymphocytic infiltrations between the follicles. There was fibrosis, but generally this was mild, and most marked interlobularly; on occasion it was evident between follicles. Colloid usually was reduced in amount and granular, or at times dense, with marginal scalloping. The intermediate lesions showed equivocal oxyphilia or epithelial infolding. Other features were similar to those just described.

The lack of oxyphilia in the cytoplasm of thyroid cells of some of the patients with struma lymphomatosa was probably a pathologic indication of a lesser degree of cellular dysfunction than when marked oxyphilia was present. The goiters characterized by lack of oxyphilia in the cells occurred in younger patients, and clinically these patients had a lesser degree of thyroid failure.

Treatment with desiccated thyroid

Since the results of this study indicate that struma lymphomatosa is primary thyroid failure with compensatory goiter, the treatment of choice in the patients in this series usually was a sufficient amount of uncoated U.S.P. desiccated thyroid. The rationale of thyroid therapy is to depress the excess production of TSH which is believed to be the cause of enlargement of the failing gland. In most instances administration of thyroid decreased the goiter significantly (Table 1), but usually not to normal size. Most of the reduction occurred within two months. Figure 2 illustrates the effect of desiccated thyroid in reducing the size of the goiter. Less marked reduction occurred in the goiters characterized by increased fibrosis (Patients 5, 14 and 18), or when an insufficient amount of desiccated thyroid was given.

The dosage of desiccated thyroid that induced the most consistent decrease in size of the goiter was 3 grains daily. If the patient could not tolerate this dose, it was reduced to 2 grains or less. Patients with struma lym-

phomatosa are definitely more sensitive to desiccated thyroid than are euthyroid patients and in a few instances clinical and laboratory signs of exogenous hyperthyroidism developed when a dosage of 3 grains daily was given (Patients 21 and 22). Even though the patient's thyroid failure be

Pre-treatment *Post-treatment*

FIG. 2. Effect of 3 grains of desiccated thyroid daily in significantly decreasing the size of the large goiter of a patient with struma lymphomatosa.

clinically compensated, desiccated thyroid should be given permanently, since no one can predict when clinical decompensation will occur. Patients are relieved of considerable apprehension concerning the goiter when they note a decrease in size after treatment. Sodium levo-thyroxine (0.3 mg. daily) was used in 3 patients and the clinical and laboratory changes were no different from those observed during therapy with desiccated thyroid.

The use of iodine should be avoided. In 1 of our patients the goiter rapidly enlarged on two occasions after she had been given Lugol's solution.

DISCUSSION

The results of these observations indicate that struma lymphomatosa is probably primary thyroid failure with compensatory thyroid enlargement and is not chronic thyroiditis. These studies also indicate that the basic defect is probably a failure of the thyroid cells, resulting in a deficiency of thyroxine. The histopathologic evidence for this failure of the thyroid cells is usually, but not always, shown by varying degrees of oxyphilia in the cytoplasm of the cells. The deficiency of thyroxine results in increased production of thyrotropic hormone in an attempt to stimulate the cells to increase their output of thyroxine. Histopathologically, this may induce hyperplasia and often hypertrophy of the thyroid cells, causing most of the thyroid enlargement. Lymphocytic infiltration and fibrosis in these goiters are probably secondary to the failure of the thyroid cells, rather than being the causes of this failure, and also contribute to a lesser extent to the enlargement of the goiter except when the hyperplasia is largely replaced by fibrosis. Due to the compensatory hyperplasia of the thyroid cells, the patient may exist in a clinically euthyroid state (compensated thyroid failure) for months to years before a clinically hypothyroid state (decompensated thyroid failure) occurs. Treatment with desiccated thyroid decreases the size of the goiter by depressing pituitary TSH production, and corrects both symptomatic and asymptomatic thyroxine deficiency.

A high postoperative incidence of clinical thyroid failure in patients with struma lymphomatosa follows removal of a sufficient amount of this hypofunctioning thyroid tissue. If the amount of tissue removed be insufficient to produce clinical thyroid failure, the residual tissue may undergo further hyperplasia under TSH stimulation and the goiter may enlarge again.

Since the goiter of struma lymphomatosa is a failing gland with compensatory enlargement, there seems little reason for surgical treatment, particularly since the patient usually has to take desiccated thyroid postoperatively anyway. Moreover, the diagnosis can now be made preoperatively.

Heretofore thyroid failure with goiter has been regarded as an entity separate from thyroid failure without goiter. Clinical and laboratory findings and histopathologic patterns (including oxyphilia of thyroid cells, lymphocytic infiltration and fibrosis) similar to those in struma lymphomatosa have been observed also in patients with primary thyroid failure without goiter (37–40). This indicates that the two conditions are probably clinical variants of the same disease. The reason for the inability of the thyroid to

enlarge in response to TSH stimulation in many patients with thyroid failure is unknown. It may be that the initial degree of failure of the individual cells determines their ability to undergo hyperplasia in response to TSH stimulation.

The cause of the failure of the thyroid cells is unknown. The only precipitating factor that could be elicited in a few of the patients in this series was the stress of pregnancy, which is known to increase the work of the thyroid gland (41).

Another possible precipitating factor, not observed in the patients in this series, appears to be overactivity of the thyroid cells in certain patients with Graves' disease, resulting in focal areas of thyroid-cell exhaustion characterized by oxyphilia, lymphocytic infiltration and fibrosis. This has also been called "thyroiditis" and has been recently reviewed by Whitesell and Black (42) who noted, as others have before them, the high incidence of postoperative thyroid failure in this particular group of patients.

CONCLUSIONS

1. In 36 patients, clinical, laboratory, pathologic and therapeutic findings indicate that struma lymphomatosa is not chronic thyroiditis, but rather primary thyroid failure with compensatory thyroid enlargement.

2. Supporting clinical evidence was furnished by the occurrence of symptomatic thyroid failure in 10 patients, and compensatory goiter in all.

3. Laboratory studies showed the predominance of low basal metabolic rates (22 of 36 patients), elevated serum cholesterol values (9 of 25 patients), low-normal serum total protein-bound iodine values (9 of 12 patients), low or low-normal serum butanol-extractable protein-bound iodine values (12 of 12 patients) and abnormal TSH-test results (18 of 18 patients). Increased serum gamma-globulin levels and usually decreased albumin levels were observed in 16 of 20 patients, and were probably due to thyroxine deficiency. This change could be measured by the serum colloidal-gold test, which yielded positive results in 20 of 25 patients. Thyroidal 24-hour radioactive iodine uptakes varied from low to high in 26 patients. The response to these new diagnostic tests indicates that the incidence of struma lymphomatosa is greater than has been previously recognized.

4. Pathologic studies of needle biopsy specimens obtained from the thyroid in all 36 patients with struma lymphomatosa showed hyperplasia of the thyroid cells, usually with oxyphilia (32 of 36) in the cytoplasm, lymphocytic infiltration and fibrosis. The similar occurrence of oxyphilia, lymphocytic infiltration and fibrosis in the thyroid glands of patients with primary thyroid failure without goiter, indicates that the two conditions are probably clinical variants of the same disease.

5. Since struma lymphomatosa can now be diagnosed accurately with-

out the use of surgery, the treatment of choice is an adequate dosage of desiccated thyroid. Such therapy significantly decreased the size of the goiter in most of these patients by suppressing thyrotropin production, and corrected both symptomatic and asymptomatic thyroid failure.

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Addendum

Since this paper was submitted for publication, it has been noted that an elevated serum gamma globulin level has been previously reported in 4 patients with struma lymphomatosa (Fromm, G. A.; Laseano, E. F., and Escanlante, E.: Chronic nonspecific thyroiditis, *Rev. Asoc. méd. argent.* **67**: 162 (May 15), 1953).

We have now studied successive pathologic specimens obtained over periods of one year and one and a half years, respectively, from the goiters of 2 other patients with untreated struma lymphomatosa. The pathologic picture in each of these patients changed from the hyperplastic to the Hashimoto type. Also, in 3 of our 30 patients with Hashimoto type goiter reported in this paper, the hyperplastic cellular tissue was extensively replaced by fibrous tissue leaving only small areas of atrophic oxyphilic cells and marked lymphocytic infiltration. If such a fibrous goiter is very large, as it was in 1 of our patients, surgery may be indicated for cosmetic reasons. No evidence of associated malignancy was observed in any of our patients.

In addition, 6 more women with proved struma lymphomatosa were observed by us. Four of the goiters were of the Hashimoto type, and 2 were of the hyperplastic type. In 3 of these 6 women, the goiter appeared within four months after parturition. The BMR was low in only 2 patients. Thyroidal I^{131} uptakes in 5 TSH tests performed on 5 patients were 53 and 60, 12 and 11, 53 and 53, 46 and 46, and 46 and 61 per cent, respectively.

The serum gamma globulin values were increased in all 6 patients, being 1.14, 1.15, 1.24, 1.47, 1.65, and 1.79 Gm. per 100 ml. The serum colloidal gold reaction was abnormal in 5 of the 6 patients; in 1, a serum albumin of 4.67 Gm. per 100 ml. inhibited precipitation of the colloidal gold by a gamma globulin of 1.24 Gm. per 100 ml. Abnormal serum colloidal gold curves also were observed in 3 more patients with proved nontoxic adenomatous goiter, in whom gamma globulin values were 1.08, 1.15, and 1.20 Gm. per 100 ml. This further indicates that the colloidal gold test will not differentiate these two types of goiter. The serum cholesterol level was elevated in only 2 of the 6 patients with proved struma lymphomatosa (252 and 265 mg. per 100 ml., respectively).

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EVALUATION OF THE PERIPHERAL BLOOD SMEAR TEST IN THE DETECTION OF CHROMOSOMAL SEX IN THE HUMAN

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IN THE past few years there has been an increasing interest in the detection of chromosomal sex by means of the morphologic differences in the various tissue cells (1, 2). In 1949, Barr and Bertram (3–5) first recorded a sex difference in the nuclear pattern of the various tissues of the cat. A similar difference has since been noted in the nuclei of the nerve cells of many other lower mammals and in the nuclei of the cells in the malpighian layer of human skin (6–10). The nucleus of the cells of females contains a special, heavy mass of chromatin measuring about 1 micron in diameter. This chromatin mass is present in most female cells and only rarely is seen in male cells. When present, the "sex chromatin" mass is usually situated along the inner surface of the nuclear membrane.

Recently Davidson and Smith (11) reported that there likewise is a morphologic sex difference in the nuclei of the mature neutrophils of humans They observed a heavy chromatin aggregation in the form of a drumstick projecting from the multilobulated nuclei. These bodies are present only in the neutrophils of the smears from females.

The present study is intended 1) to evaluate this peripheral blood film test in a group of normal males and females, and 2) to attest its clinical value as an additional laboratory aid in the differential diagnosis of chromosomal sex in certain perplexing problems of intersexuality.

MATERIAL AND METHOD

Two hundred and thirty-four human peripheral blood smears were used in this study. They could be divided into three groups:

Group A consisted of 20 smears taken from normal males and females aged from 14 months to 64 years. The sex of the subjects was made known to the examiner and these smears were used initially for studying the morphologic difference between the cells in the two sexes, as reported by Davidson and Smith.

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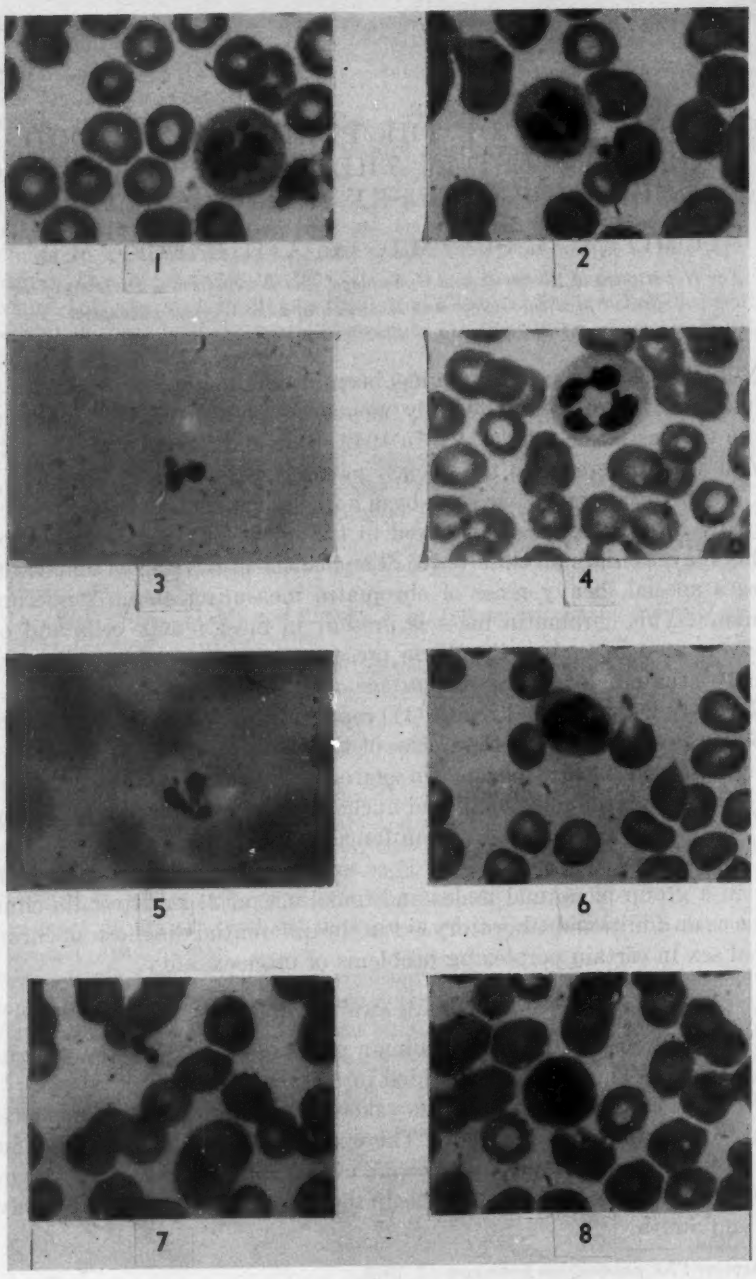
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Group B consisted of 200 smears obtained from normal males and females. In these instances, the sex of the subjects was not known to the examiner. The ages in this group ranged from a few hours after birth to 70 years. Included were 10 normal pregnant women.

Group C consisted of smears from 14 patients available to us during the period of this study, in whom there were manifestations of intersexuality.

The technic of the peripheral blood film test is relatively simple when compared to skin biopsy (6, 10, 12) and tissue culture methods (13). A thin, evenly distributed smear on a slide is as satisfactory as a smear on a coverglass. Too thin a smear, although desirable for many other hematologic studies, is not particularly suitable for this test. When completely dry, the slides are fixed in absolute methyl alcohol for ten to fifteen minutes, and are again allowed to dry at room temperature. The combined May-Grünwald-Giemsa staining method, which is described in many of the hematology textbooks (14) was used for smears from all subjects. Additional smears from a few of the subjects were treated by means of the Feulgen reaction (15), whereby the sex chromatin bodies are stained red.

At least 6 polymorphonuclear neutrophils showing the characteristic chromatin body ("drumstick") are required before a smear can be classified as of female origin. After examining 500 consecutive mature neutrophils without observing a "drumstick," the smear is then classified as of male origin. "Small clubs," "rackets," and small "sessile nodules" occur in about the same proportions in the cells of both sexes, and are not to be confused with typical "drumsticks" (Figs. 1–8).

RESULTS

Group A. In this initial group of blood smears, taken from 13 females and 7 males, we were able to confirm the observation of Davidson and Smith. In the smears from females, the number of polymorphonuclear neutrophils counted before 6 drumstick chromatin bodies were found,

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FIG. 1. Typical "drumstick" chromatin mass in female blood smear. Note the homogenous, dense appearance of the chromatin aggregation.

FIG. 2. "Sessile nodule," usually found in female blood smear, protruding from one side of the bilobular nuclear. Note its close resemblance to the head of a "drumstick."

FIG. 3. The positive Feulgen reaction of a typical "drumstick."

FIG. 4. "Small club" and "small sessile body." These are seen in both male and female smears.

FIG. 5. The negative Feulgen reaction of a "small club."

FIGS. 6 and 7. Small nuclear lobes of various sizes, not to be mistaken for "drumsticks." Note the connecting thin strands of chromatin material.

FIG. 8. "Racket," with a pale hollow center, often present in the male blood smear.

ranged from 48 to 246. No such chromatin bodies were observed in any of the smears from males in the examination of 500 consecutive neutrophils.

Group B. In this group of 200 blood specimens, in which the genetic sex of the subject was not known to the examiner, 133 were from females and 67 from males. Our readings were correct in all of these smears, using the described technique. In the smears from females, the number of neutrophils counted before 6 drumstick chromatin bodies were found, ranged from 60 to 450 (Table 1). In none of the smears from males was such a chromatin body observed in 500 consecutive neutrophils.

Group C. Problems of intersexuality (Table 2).

1. Gonadal Agenesis. Among the 7 patients with "ovarian agenesis,"

TABLE 1. DISTRIBUTION OF THE FINDING OF 6 "DRUMSTICKS" IN 133 UNKNOWN NORMAL FEMALE BLOOD SMEARS

No. of PMN* counted in order to find 6 "drumsticks"	No. of smears	Percentage
50 to 100	12	9
101 to 200	47	35
201 to 300	37	28
301 to 400	24	18
401 to 500	13	10
	Total 133	*100%*

* Polymorphonuclear neutrophils.

6 showed blood smears of the male type and 1 showed a smear of the female type.[1] These patients ranged in age from 14 to 29 years. All had stigmata of primary hypogonadism and high urinary gonadotropins. All were short in stature except 1, who was of normal height (5 feet, 4 inches) with normal skeletal proportions; the diagnosis in this case was confirmed by laparotomy.

2. Pseudohermaphrodism. There were 5 cases of pseudohermaphrodism. In the 3 cases of female pseudohermaphrodism due to congenital adrenal hyperplasia, the blood smears showed 6 drumstick chromatin bodies distributed in 92, 234 and 249 neutrophils, respectively. In the 2 cases of male pseudohermaphrodism, no such chromatin masses were visible.

3. True Hermaphrodism. The blood smear obtained in a case of questionable true hermaphrodism was unfortunately too thin. Examination of 392 neutrophils failed to reveal a "drumstick."

[1] Since this paper was submitted, we have examined 8 additional patients with gonadal agenesis, making a total of 15; of these, 13 (86.6 per cent) had male type smears.

TABLE 2

Group	No. of smears	Chromosomal sex		Accuracy
		Male	Female	
Group A:				
Normal subjects.				
Sex known.	20	7	13	100%
Group B:				
Normal subjects.				
Sex unknown.	200	67	133	100%
Group C:				
Pseudohermaphrodites	5			
male		2		100%
female (adreno-genital syndrome)			3	100%
True hermaphrodite?	1	1?		
Ovarian agenesis*	7	6	1	
Stein-Leventhal syn.	1		1	

* *See* text, footnote 1.

DISCUSSION

The reflection of maleness and femaleness in the chromosomal content of specialized somatic cells has long been observed and emphasized by the geneticists. Severinghaus in 1942 (16) for the first time was able to examine the sex chromosome pattern XY in the male germ cell during meiosis in a case of human intersexuality.

Although direct proof is still lacking, there is a great deal of evidence at present to indicate that the heavy chromatin mass (the so-called "sex chromatin") found in the female cell is derived from sex chromosomes. The high desoxyribose nucleic-acid content of this chromatin is demonstrated in the positive Feulgen reaction, thus relating it to the sex chromatin which also contains nucleic acid of desoxyribose type. The ability of certain portions of the sex chromosomes to remain compact in the nucleus during meiosis is known to be more intense, as compared to the autosomes. Barr postulated that when the heterochromatic portions of two X chromosomes combine to form a single chromatin mass, the mass becomes sufficiently large to be identified readily. In the case of the male cell, the Y chromosome makes a much smaller contribution, so that the XY chromatin mass is much smaller or indistinct. In rodents, the Y chromosome is rather large, and thus there is little difference in the size of this special chromatin mass between male and female cells (17).

Experimental neurocytologists have noted that during the period of intense ribose nucleoprotein synthesis or altered cellular metabolism, this special chromatin mass increases in size and tends to move away from the nucleolus towards the nuclear membrane (4, 18).

Davidson and Smith believe that, in the human, the chromatin aggregation in the solitary "drumstick" represents the XX chromosomes corresponding to the "sex chromatin" observed in the tissues. In the case of XY combination, the aggregation of chromatin results in an insignificant projection in the form of a "club," "racket," or "small nodule," or remains insignificant. It will be of interest to observe whether a similar morphologic sex difference is present in the blood smears of any other mammals.

The application of this peripheral blood smear method as an added laboratory procedure in the diagnosis of various types of intersexuality is still in a preliminary stage. Our results in the 5 cases of proven pseudohermaphrodism were encouraging. The smear obtained in the case of questionable true hermaphrodism, unfortunately, was unsatisfactory; it was temporarily classified as of the male type on the basis of the absence of drumstick chromatin bodies in the 392 neutrophils which were counted. In the 7 cases of ovarian agenesis, diagnosed prior to examination of the blood, 6 of the smears were of the male type and 1 was of the female type (19, 20). In these cases it is our intention to examine skin biopsy specimens in the future. The establishment of chromosomal sex in children suspected of ovarian agenesis may be helpful in making the diagnosis with certainty at an early age, if the blood smear is of the male type. This procedure may be particularly useful if the child be in the pubertal years when a high gonadotropin level is of less significance than in early childhood. Thus far, our study indicates that the chromosomal sex of an individual is revealed as definitely in the neutrophils as it is in the somatic cells. It is our hope that this brief preliminary report may encourage more interest in the use of this relatively simple method in the differential diagnosis of intersexuality.

SUMMARY

1. A study of 234 human peripheral blood smears, stained by the May-Grünwald-Giemsa and Feulgen methods, is reported.

2. The observation made by Davidson and Smith that there is a definite morphologic sex difference in the nuclei of the polymorphonuclear neutrophils is confirmed.

3. The peripheral blood smear test has been applied in 14 cases of intersexuality. Preliminary study indicates that this simple test has definite clinical value in the differential diagnosis of certain types of intersexuality.

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HYPERTHYROIDISM IN CHILDREN: OBSERVATIONS IN 50 TREATED CASES, INCLUDING AN EVALUATION OF ENDOCRINE FACTORS*†

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INTRODUCTION

HYPERTHYROIDISM (Graves' disease) in children is rare, the incidence being from 1 to 2.5 per cent of all cases of Graves' disease. Atkinson (1) reported 208 cases, Sattler (2) 184 cases, Kennedy (3, 4) 171 cases, and Bram (5) 128 cases; many other have reported smaller series of cases. Few have given any detailed follow-up information.

The present report concerns 50 children ranging in age from 2 to 15 years, with thyrotoxicosis, who were observed in the Albany Hospital from 1918 to April 1955. In 75 per cent of the cases there was a clinical follow-up, which provided an unusual opportunity to evaluate the clinical manifestations of this disease, its course and its effect on the body's maturative processes. Important information regarding treatment was also obtained. The clinical characteristics of juvenile thyrotoxicosis vary so greatly that only through a wide experience with large numbers of such patients is there an opportunity to further our knowledge of this disorder significantly and thereby to contribute to better diagnosis and treatment. The clinical similarity between the juvenile and adult forms of thyrotoxicosis, mentioned by many authorities, was also observed in the present series. Early recognition and treatment should be emphasized.

In reviewing the cases which form the basis for this report, it was noted that in some respects the widely quoted effects of juvenile hyperthyroidism on growth, development and sexual maturation could not be substantiated.

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The endocrine status was determined and evaluated in most of the 50 patients. The long-term observations in such a large group, all treated in much the same manner, are unique in the recorded literature of juvenile thyrotoxicosis.

CLINICAL MATERIAL

The medical records of 50 patients under 15 years of age were reviewed. An upper age limit of 15 years was established because it is considered generally to represent the upper limit for a normal onset of sexual maturation. The authors were personally acquainted with 35 of the patients, having participated either in establishing the diagnosis or in the treatment program. All lived within a 75-mile radius of Albany. Eight patients have been mentioned in three previous reports (6–8).

The frequency with which hyperthyroidism was diagnosed at the Albany

TABLE 1.

FREQUENCY OF JUVENILE THYROTOXICOSIS AT ALBANY HOSPITAL 1915-1955

1915-1919	1920-1924	1925-1929	1930-1934	1935-1939	1940-1944		1945-1949	1950-1954	1955—
1	2	6	7	10	7	33	7	8	2

Hospital in children under 15 years of age is summarized in Table 1. From the year 1918 through the year 1944, 33 such cases were diagnosed and treated. In the following ten years (1945 to 1955) over half as many were observed as in the first 27-year period.

Ideal and complete follow-up was difficult to attain. Adequate information was procured in 37 of 49 living patients. Most of these were interviewed and examined personally by at least one of the authors, but in a few instances the data were obtained by telephone communication or by letter. Fortunately, the information included complete menstrual, marital and pregnancy histories in all the female patients. Serum cholesterol and protein-bound iodine levels were determined in 23 patients. Photographic records of each case were made at the time of observation (Fig. 1).

The duration of follow-up in some of the cases was noteworthy. Eight patients were followed for twenty to thirty-seven years, 7 for fifteen to twenty years; 9 for ten to fifteen years and 6 for less than ten years but more than five years following diagnosis and treatment (Fig. 2).

The ages and sex distribution of these patients are presented in Table 2. It is of interest that the youngest child was 2¾ years old at the time of diagnosis and her first operation; 2 others were seen at 4 years of age and a fourth began medical treatment at 5 years of age. There were only 2 males; thus the female-to-male sex ratio was 24:1.

FIG. 1. **A.** Patient *J.S.*, aged 2 years 9 months, prior to her first operation. Note thyroid enlargement.
B. Patient *J.S.*, aged 7 years. Note difference in palpebral fissures.
C. Patient *J.S.*, aged 13. Difference in eyes now less apparent.
D. Patient *J.S.*, aged 26, twenty-four years after first definitive treatment for thyrotoxicosis. Note how few eye signs remain.

MANAGEMENT

With few exceptions all patients were treated surgically. This was due to the widely-held opinion that surgery was the treatment of choice and to the unavailability in former years of an adequate form of medical therapy. Three of 6 children in whom a diagnosis of hyperthyroidism was made during the past year, received goitrogenic therapy.

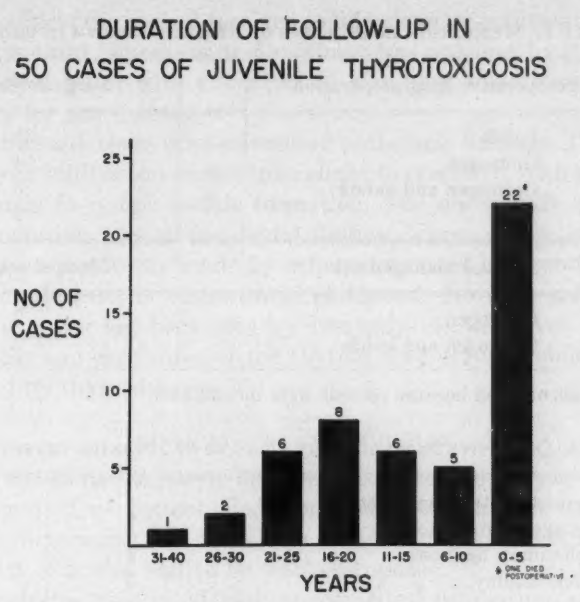

FIGURE 2

Two patients were treated prior to the popularization of iodides as a form of medical or preoperative therapy. Rest, sedation and a high caloric diet constituted the basic program in all patients; 30 patients were prepared preoperatively with iodides in addition (Table 3).

Forty-five patients underwent some form of surgical procedure. One patient required preliminary ligations of the superior thyroid arteries before subtotal thyroidectomy. In a second, subtotal resection of the thyroid was carried out in two stages. All others had a one-stage bilateral subtotal thyroidectomy (Table 4). Among the 20 patients in whom the diagnosis was made since the advent of goitrogenic drugs, 19 received one or

TABLE 2. AGE AND SEX DISTRIBUTION IN 50 CASES OF HYPERTHYROIDISM IN CHILDREN

Age (yrs.)	Males	Females	Total
Under 6	0	4	4
6–10	1	11	12
11–15	1	33	34
Total	2	48	50

TABLE 3. MEDICATION IN 48* CASES OF HYPERTHYROIDISM IN CHILDREN

Preoperative drug preparation	Surgical remission
Iodide	31
Goitrogen	4
Goitrogen and iodide	6
None	2
Medical management	**Medical remission**
Goitrogen	2
Goitrogen and iodide	3

* Two cases omitted because records were incomplete.

TABLE 4. OPERATIVE PROCEDURES IN 45 CASES OF JUVENILE THYROTOXICOSIS

Bilateral subtotal thyroidectomy		42
Two-stage thyroidectomy		1
Preliminary ligations	1	
Tracheostomy	2	
Unknown		2

more of these compounds, and 1 received iodides only. Of the group receiving goitrogens, 1 patient has been free from symptoms of hyperthyroidism for forty-two months after discontinuing antithyroid therapy. Three children are currently receiving these drugs.

THYROID PATHOLOGY

The histopathology of the thyroid glands removed in 44 cases clearly indicates the problem under discussion (Table 5). Forty-one showed hyperplasia. Three patients were found to have colloid goiter and are worthy of some comment. One was treated for twenty-one months with goitrogenic

TABLE 5. HISTOPATHOLOGY OF THYROID GLAND IN 45 CASES OF JUVENILE THYROTOXICOSIS

Hyperplasia		41
Colloid goiter		3
Unknown		1
Associated findings		
Nodule	1	
Lymphoid infiltration	10	

drugs and iodides; the second received iodide alone intermittently for four years; and the third (whose surgical specimen was classified by the pathologist as a colloid goiter with a few foci of glandular hyperplasia) had iodide therapy for one month.

In 11 of the cases there were associated pathologic findings. The degrees of lymphocytic infiltration varied from slight to excessive, with a tendency in some glands to lymph-nodule formation. The one nodule undergoing cystic degeneration was an incidental finding. These results corroborate the observation previously made by others interested in hyperthyroidism —that toxic adenoma is unknown in childhood. No such case of toxic adenomatous goiter has been seen by Kennedy (4). However, both nontoxic adenoma and carcinoma of the thyroid may occur in childhood, unaccompanied by thyrotoxicosis.

COMPLICATIONS AND SEQUELAE

Thirty of the 45 patients were operated upon prior to the use of antithyroid compounds. One patient died from pneumonia in this early period when chemotherapeutic and antibiotic drugs were also unavailable. This patient was 1 of 2 who had to be tracheostomized. In the second patient technical problems associated with endotracheal intubation necessitated the tracheostomy.

Hypothyroidism was the most frequent complication observed (Table 6). It occurred in 13 patients, 4 of whom definitely recovered; in the other 9 no definite information relative to their present state of thyroid function was available. Some of these patients were known to have continued taking thyroid after operation, but it was not known whether such medication was still necessary. Because the medical supervision of many of the children in

TABLE 6. INCIDENCE OF SURGICAL COMPLICATIONS AMONG 45 CASES OF JUVENILE THYROTOXICOSIS FOLLOWED MORE THAN ONE YEAR

	No. of cases	Recovered under treatment
Hypothyroidism	13	4
Hypoparathyroidism	5	1
Persistent exophthalmos	6	
Re-operation for recurrence	4	
Progression of exophthalmos	1	
Auricular fibrillation	1	
Death	1	
Keloid	0	

this series was not the responsibility of any of the authors, it was particularly difficult to evaluate this aspect of the management. In the patients who were examined by us there was no clinical evidence of hypothyroidism, and the results of chemical analysis of the blood did not suggest this diagnosis (Table 7).

Clinical evidences of hypocalcemia, presumably due to hypoparathy-

TABLE 7. CURRENT* SERUM CHOLESTEROL AND PROTEIN-BOUND IODINE LEVELS IN 23 PATIENTS WHO ORIGINALLY HAD JUVENILE HYPERTHYROIDISM

Patient and age (yrs.) at time of diagnosis	Present age (yrs.)	Years since initial treatment	Current therapy	Serum cholesterol (mg/100 ml.)	Serum PBI (μg./100 ml.)
M.L.—11	37	26		194	5.6
A.P.F.—12	35	23	Thyroid, 60 mg.	185	
M.Y.B.—13	36	23		246	7.2
J.S.—2	27	23	Thyroid, 60 mg.	164	6.4
M.R.—11	33	22	NPH insulin	232	7.2
E.S.—13	31	18		177	6.4
B.G.—4	23	18	Thyroid, 90 mg.	246	6.0
B.O.—14	31	17		167	4.4
M.M.—7	27	16		218	4.4
H.R.—14	32	14		236	6.4
M.T.—14	39	14		169	10.0
S.P.—12	25	13		182	6.4
E.V.—8	25	13		217	5.6
M.G.—7	20	13	Vitamin D	154	5.6
J.H.—9	19	10	Thyroid, 90 mg.	191	6.0
N.F.—14	23	9		271	7.6
D.W.—14	20	6		182	5.6
B.H.—9	16	6	Thyroid, 30 mg.	133	3.6
S.D.—14	19	5	Thyroid, 60 mg.	184	4.0
G.McR.—9	14	5		101	12.6
P.F.—11	16	5		190	6.0
B.H.—15	18	3	Thyroid, 60 mg.	237	5.2
M.R.—14	15	1		143	8.0

* Year 1955.

roidism, were observed in 4 patients (Table 6). One recovered completely; the others are receiving calcium and vitamin D, with a satisfactory response.

Twenty-six patients had bilateral exophthalmos at the time of diagnosis of hyperthyroidism. Twenty-two underwent thyroidectomy, but in 6 there was no improvement in exophthalmos. One patient showed progression of

exophthalmos, which then became stationary and did not require neurosurgical intervention.

Four patients underwent a second operation for recurrence of hyperthyroidism, but no additional complications were observed as a result.

Other complications were infrequent and are listed in Table 6. A postoperative thyroid storm was observed in some of the earlier cases, but with present-day improved preoperative preparation this complication is exceedingly rare.

CLINICAL ASPECTS OF JUVENILE THYROTOXICOSIS

The recognition of hyperthyroidism in children is generally easy, but may present some difficulties early in its course. This is due to the fact that other associated changes such as growth, emotional problems and infection may so dominate the picture that the true nature of the basic thyroid disturbance may be unrecognized for a long period of time. Nevertheless, the thyroid manifestations are classic and should be sought for in all questionable cases.

Incidence and age distribution

Lyons (9) in a study of juvenile hyperthyroidism reported its occurrence in 8 children among 78,659 admissions to the Children's Hospital, Washington, D. C.—an incidence of 0.01 per cent. Among all cases of Graves' disease, the number of children affected ranges between 1 and 2.5 per cent. Sattler's (2) extensive report of 3,477 cases of exophthalmic goiter included 184 patients under 15 years of age. Bloom (10) commented on the infrequence of hyperthyroidism among Jewish children and stated that only 2 cases had been observed in Negro children. The same author reported in 1935 that juvenile hyperthyroidism is increasing; and now, twenty years later, we have arrived at the same conclusion based on our series of 50 patients. The diagnosis has been made in 17 patients in the past ten years compared with 33 patients in the preceding thirty years. The reason for this is not apparent, but it includes an increasing awareness of, and availability of more accurate indices of, thyroid function.

Hyperthyroidism is found at all ages. White (11) described hyperthyroidism in a male fetus and Margett (12) reported an instance in a 10-week-old male infant. Only 6 cases in infants under the age of 1 year have been reported. In our series the youngest child was $2\frac{3}{4}$ years of age and 3 other patients were under 6 years of age (Table 2). In 7 to 8 per cent of children hyperthyroidism develops under the age of 5 years. Atkinson (1) reported an unusually high incidence of 25 per cent in this age group. The age distribution in the present series is compared below with averages obtained from the literature:

Age (yrs.)	Other series	Present series
0–5	10%	8%
6–10	30%	24%
11–15	60%	68%

In the age group 11–15 years, there were 34 patients, or more than twice the total number in the 0–10 year group. The increased incidence during pre-puberty and puberty has been observed repeatedly. It is generally recognized that about 75 per cent of cases of hyperthyroidism occur in patients under 15 years of age.

Etiology

It is as difficult in children as in adults to determine the factors which play a significant role in the development of the hyperthyroid state. Heredity plays some role, as shown by the 11 cases in one family reported by Boas and Ober (13). This was, however, an unusual situation; in general, heredity is not of great importance, although it is difficult to separate hereditary factors from environmental factors that may be casually related to hyperthyroidism. Bram (5) observed that in his series of 128 patients 50 per cent were related to infections such as scarlet fever, measles or pertussis, and only 6 per cent to psychic trauma. The latter figure differs markedly from that observed in adult hyperthyroidism. Edgren (14) also felt that infection was a frequent antecedent of juvenile hyperthyroidism. However, Dinsmore (15) stated that in only a few of his cases was there an antecedent infection, and in only 3 of Greene and Mora's (16) 26 cases was there such a history. Kennedy (3, 4) emphasized the importance of psychologic maladjustments in children and attached primary importance to dealing with the psychologic factors in any successful approach to the treatment of juvenile hyperthyroidism. Seckel (17) observed a case of hyperthyroidism due to a presumed thyrotropic pituitary adenoma, but such reports are extremely rare.

In only 14 per cent of our 50 patients was the precipitating cause determined—infection being the most common. It might be inferred that psychic factors existed in the remaining 86 per cent of the cases, but no specific information was available to substantiate this possibility. The increasing tendency in the last decade to seek a psychosomatic basis for diseases such as nonadenomatous hyperthyroidism would undoubtedly result in an increase in the percentage of patients having preceding psychic trauma above the 6 per cent quoted by Bram (5). Nevertheless, although fluctuations in the endocrine equilibrium are more dynamic in children and may, therefore, be a primary rather than secondary cause of thyroid overactivity, the

role of psychic factors may be as important as in adults and should not be overlooked.

Symptoms

The symptoms are in the main identical with those observed in the thyrotoxic adult, but there are specific differences. There is also some difference in the incidence of certain symptoms. In Table 8 the frequency of symptoms in our 50 patients is recorded. In over 33 per cent of the cases, four of the six most frequent symptoms were subjective, *i.e.*, nervousness, fatigability, irritability and emotional disturbances. Greene and Mora (16) compared the symptoms of 26 thyrotoxic children with those of a large series of adult patients and noted that the ten most prominent symptoms in

TABLE 8. FREQUENCY OF SYMPTOMS IN 50 CASES OF JUVENILE THYROTOXICOSIS

Symptoms	Per cent of cases
Nervousness	78
Fatigability	58
Enlarged thyroid	52
Irritability	46
Increased appetite	46
Emotional disturbance	34
Weight loss	34
Sweating	32
Palpitation	30
Heat intolerance	28
Restlessness	24
Dyspnea	24
Subjectively hot	18
Dysphagia	14
History of precipitating factor	
Infection 10%	
Surgery 2%	
Trauma 2%	
Rapid growth	12
Altered menses	4
Headache	4
Insomnia	4
Failure to gain weight	4
Anorexia	4
Diarrhea	4
Itching	2
Family history	2
Cough	2

the adults and the children were the same. The incidence differed somewhat, however. Generally, too much reliance should not be placed on the exactness of percentages because, at least in younger children, the information regarding symptoms is usually obtained from the parents and is therefore subject to their interpretative errors. Greene and Mora (16) reported palpitation in 30 per cent of their series, which is identical with the incidence in our series; irritability in 23 per cent, compared to 46 per cent in ours; restlessness in 19 per cent, compared to 24 per cent in ours; and nervousness in 88 per cent, compared to 78 per cent in our series. Bloom (10) reported the presence of nervousness in 95 per cent of his 40 cases. It would appear that there is fairly close agreement as to the frequency of many symptoms when different series of patients are compared.

Greene and Mora (16) reported weight loss in 57 per cent of their cases, Helmholz (18) in 53 per cent, and Bloom (10) in 82 per cent; but in our series, weight loss occurred in only 34 per cent. These discrepancies are not unusual, particularly in children, in whom the processes of skeletal growth (whether normal or accelerated) may affect any apparent weight loss. One patient of Greene and Mora's (16) showed a gain in weight. Talbot (19) states that, because of increased appetite, there may be no weight loss in thyrotoxic children.

The fact that 52 per cent of the children in the present series were brought to a physician because of an enlarged thyroid is particularly interesting. This emphasizes that the change in thyroid size is of such magnitude that it may be noticed even by lay persons.

Dyspnea occurred in 24 per cent of cases. This was considered a rather unusual finding, until it was noted that Helmholz (18) had reported it in 40 per cent, and Dinsmore (15) in 55 per cent of their cases. This symptom may be overlooked in children because of their boundless energy and inexhaustible activities, which may normally be expected to produce some dyspnea or exhaustion.

Many other symptoms are listed in Table 8. Rapid growth was mentioned by only 12 per cent of patients. Alterations in menstrual pattern occurred in only 4 per cent. These two changes are discussed more fully subsequently. The infrequency of diarrhea and other gastro-intestinal manifestations is noteworthy.

In summary, the symptoms of juvenile thyrotoxicosis do not differ from those of the disease in adults. Central nervous system disturbances account for most of the symptoms in children, whereas cardiovascular disturbances predominate in adults. The recognition of symptoms in children is generally difficult because of their inability to appreciate slight alterations in their physiologic make-up.

Signs

In the 50 children of this series the signs of thyrotoxicosis were, like the symptoms, similar to those of adult hyperthyroidism (Table 9). Cardiovascular signs, except tachycardia, were less frequent than in adults. An elevated pulse pressure was obtained in only a few patients. Auricular fibrillation occurred in only 1 child—postoperatively. An enlarged thyroid was found in 80 per cent of the cases, which agrees well with

TABLE 9. FREQUENCY OF SIGNS IN 50 CASES OF JUVENILE THYROTOXICOSIS

Sign	Per cent of cases
Enlarged thyroid	80
Tachycardia	54
Exophthalmos (bilateral)	52
Elevated BMR*	36
Tachypnea	28
Tremor of outstretched fingers	28
Diaphoresis	26
Loss of weight	24
Warm skin	22
Stare	18
Thyroid bruit	14
Excessive growth	14
Lid lag	12
Myopathic disturbance	8
Thyroid thrill	4
Auricular fibrillation	2
Precocity	2
Exophthalmos (unilateral)	2

* Test not performed in all subjects.

84 per cent in Greene and Mora's (16) series and 82 per cent in Bloom's (10). A thyroid bruit was recorded in only 14 per cent of cases; this is lower than the 70 per cent incidence in Helmholz' (18) series and the 65 per cent incidence in Bram's (5). Similarly, the incidence of tremor was much less, being 28 per cent as compared to 57 per cent in Helmholz' cases and 70 per cent in Greene and Mora's.

Of the variety of eye disturbances in these children, the most frequently noted was bilateral exophthalmos, which was present in 52 per cent of the patients. Greene and Mora (16) recorded the presence of exophthalmos in 80 per cent of cases, whereas Bloom (10) observed it in only 30 per cent. At the other extreme is the observation by Bram (5) that goiter and exoph-

thalmos occurred in 90 per cent of his 128 cases. The exophthalmos is generally reversible with treatment. In 6 of our patients it persisted, but in only 1 did it progress. Greene and Mora reported that in 21 patients with exophthalmos before operation, the eyes became normal postoperatively in 9; there was no improvement in 10; and in 2 exophthalmos persisted.

Increased linear growth was observed in 14 per cent of our cases. This is apparently less than that mentioned by other authors, but few give any precise figures. It does not support the suggestion that such an increase in growth indicates that growth activity and thyrotropic activity are closely related. The whole concept of skeletal changes in thyrotoxicosis is difficult to evaluate, because presence or absence is related directly to the duration of the thyrotoxic state. Children with disease of less than six months' duration are not likely to show any growth disturbances, although Reilly (20) suggested that growth disorders actually often precede the symptoms of hyperthyroidism. He observed that 36 of 44 patients were taller than normal at the time of diagnosis. Talbot (19) holds the opinion that thyrotoxicosis occurs in children growing rapidly; it does not cause increased growth.

ENDOCRINE ASPECTS OF JUVENILE THYROTOXICOSIS

The numerous types of glandular interplay which exist in the human being are manifested to the greatest degree in children. The duration of the growth process, the transition from childhood to adolescence and the accompanying secondary changes are dynamic biologic developments which depend upon a smoothly working and coordinated endocrine system. The development of thyrotoxicosis disturbs this orderly process. There is no agreement as to whether the abnormal thyroid state accounts for the changes that occur, or whether the hyperthyroidism is simply an accompaniment of an already disorganized endocrine state. An analysis of some of the endocrine alterations observed in the present series may throw some light on this perplexing situation.

Hypothalamus

The recent evidence that a portal venous system exists (21) between the hypothalamus and pituitary, which transports a hypothalamic hormone, may have an important bearing on any explanation of the cause of juvenile thyrotoxicosis. It is known that, in juvenile thyrotoxicosis, central nervous system disturbances account for most of the symptoms, in contrast to the cardiovascular disturbances which predominate in the adult form of this disease. Throughout the period of growth and development, hypothalamic function remains carefully controlled and its pituitary-regulating effect

delicately balanced. If the hypothalamus becomes overactive or disturbed by some irritative local focus such as a tumor, there is an alteration of its activity, and the pituitary escapes from hypothalamic inhibition. It may discharge excessively, or it may become suppressed even further. The most classic examples are constitutional precocious puberty and the precocity which accompanies tumors in the hypothalamus or adjacent areas.

The hypothalamus is influenced by cerebral cortical factors. The frequency of emotional disturbances, anxiety states and psychologic maladjustment preceding or accompanying thyrotoxicosis suggests that these factors may be acting through the hypothalamus to produce a hypothalamic-pituitary-thyroid disturbance. However, there is little clinical evidence of hypothalamic disturbances in juvenile thyrotoxicosis with which to substantiate such an hypothesis. Nevertheless, the idea is an attractive one and experimental evidence obtained in animals strongly suggests an important linkage between the hypothalamus and thyroid gland.

Pituitary

There are no known characteristic pituitary changes in juvenile hyperthyroidism. Seckel (17) described a case of thyrotoxicosis in a 7-year-old boy with a suprasellar tumor. The specimen of thyroid obtained at thyroidectomy showed parenchymatous hyperplasia. The patient was still living at the time of the report, and no observations had been made on pituitary tissue. Seckel reviewed the literature for similar cases and found that Erdheim in 1910 described a case of Graves' disease associated with a basophilic adenoma of the anterior pituitary (22). Peters (23) in 1934 reported a case of Graves' disease in an 8-year-old girl with a slowly developing intrasellar tumor. Seckel concluded that his case and that of Peters represented primary adenomas of the anterior pituitary gland which were overproducing thyrotropic hormone—"thyrotropic adenoma" of the pituitary. Until more detailed histologic studies are available, these impressions remain conjectural.

Reilly (20) has been a strong advocate of hyperpituitarism as an etiologic factor in thyrotoxicosis. Of 44 patients, 36 were considered tall for their ages. Also those who reached adulthood after having thyrotoxicosis in childhood had, in his opinion, tall and heavy skeletons. We consider growth hormone and thyrotropic hormone excretions to be closely related. Graves' disease occurs during periods of greatest growth activity (*viz.*, puberty and pregnancy), which are therefore periods of overproduction of growth hormone and possibly of other pituitary hormones such as thyrotropin. Holmgren (24) observed gigantism and acromegaloid features in juvenile hyperthyroidism, and many patients with acromegaly manifest hyperthyroidism with accompanying thyroid hyperplasia.

There is a considerable body of evidence which points to an acceleration of growth in children and adolescents with Graves' disease. In Holmgren's (24) series, 80 per cent of patients less than 20 years old were oversized for their age. Cattell (25) mentioned the early appearance of ossification centers and an increase in bone age in those with hyperthyroidism of six months' duration or longer. Bram (5) noted an augmented height in his patients. On the other hand, Olesen and Schiodt (26) noted no differences in height when their patients were compared to normal children of the same age. Schlesinger (27) observed accelerated skeletal development in children given an overdosage of thyroid. In our series, only 14 per cent of the patients showed excessive growth. Increased bone age was observed in a few cases. Advanced dental eruption was observed by Crile and Blanton (28) and by Reilly (20).

It may be concluded that, in general, accelerated growth and development may occur only if the hyperthyroidism has existed for a sufficient time—probably at least six months. Even those children who grow more rapidly during hyperthyroidism do not as a group attain ultimate heights that are greater or less than the average.

The occasional delay in maturation of secondary sex characteristics in both male and female children with thyrotoxicosis suggests that there may be some element of hypopituitarism rather than hyperpituitarism in this disease. This phase of gonadal activity is discussed more fully subsequently.

In summary, the evidence for alteration in pituitary function in hyperthyroidisim is not conclusive. However, there are apparently many endocrine changes in these children which point to the pituitary as a possible source. The pituitary-thyroid interrelationship is a dynamic one, and it is highly probable that disturbances in one are accompanied by disturbances in the other.

Parathyroids

Alterations in parathyroid activity are almost exclusively related to operative treatment of hyperthyroidism. Hypoparathyroidism developed in 4 of our patients postoperatively (Table 6), and in 1 there has been complete recovery. If recognized and treated adequately, this complication gives little trouble.

Changes in bone were observed by Reilly (20), who noted a decreased density of the bones of hyperthyroid children. In view of our present knowledge of bone metabolism, the most likely explanation is on the basis of changes in the protein matrix of bone and negative nitrogen balance secondary to thyroid overactivity, rather than to any parathyroid disturbance.

Thyroid

The status of thyroid function following treatment for hyperthyroidism in children has not been recorded frequently. Some patients have a complete remission of thyroid overactivity following simple conservative treatment and do not require any antithyroid drug or surgical therapy. However, these cases are distinctly uncommon. Other patients can be treated by one or other of these two methods and have complete recovery, provided no serious complications have developed. Hypothyroidism developed postoperatively in 13 patients (Table 6). Four of these subsequently were restored to a euthyroid state and required no further thyroid hormone.

Determinations of serum cholesterol and protein-bound iodine were made, in order to evaluate the present level of thyroid function (Table 7). The serum cholesterol determinations in 23 patients were within normal limits. The protein-bound iodine level was altered in 3 patients. In 2, it was elevated to 10.0 and 12.6 micrograms per 100 ml.; neither patient had clinical evidence of hyperthyroidism. In the third case, the protein-bound iodine level was slightly below normal—3.6 micrograms per 100 ml. This patient was already receiving 30 mg. of desiccated thyroid daily. It is particularly interesting to note the number of years that have elapsed since treatment in many of the cases. Only 7 have been followed less than ten years. All others are maintaining a normal state of thyroid activity, as long as twenty-six years after treatment. Other patients in the series have been followed longer and are also euthyroid, but it was not possible to carry out chemical determinations.

Determinations of serum protein-bound iodine concentration and thyroidal uptake of radioactive iodide have added a great deal to the evaluation of hyperthyroidism in children. The basal metabolic rate has not proved to be satisfactory in this group of young patients. Thirty-six per cent of our patients had an elevated basal metabolism test, but this test was not performed in all subjects.

Thyroid enlargement was noted in 80 per cent of cases (Table 9). Fifty-two per cent of these patients went to their physicians complaining of goiter. This finding is of major significance in evaluating the presence of hyperthyroidism. All young patients show diffuse hyperplasia. Toxic adenomas do not occur in childhood, although nontoxic adenomas are found.

The eye signs in juvenile thyrotoxicosis are similar to those observed in adults, but the severe eye changes described in adults are uncommon. In most cases, the eyes become normal promptly after treatment of the hyperthyroidism is initiated.

Adrenal medulla and cortex

Ever since the early studies of Marine and Baumann (29) the question of a thyroid-adrenal relationship has been considered. Many of the symptoms of thyrotoxicosis are similar to those accompanying an excess of adrenal medullary hormone. It is conceivable that the immature nervous system of the child reacts more intensely to epinephrine than does the nervous system of the adult. This may be one other reason for the predominance of nervous system disturbances in juvenile thyrotoxicosis.

Little is known about the function of the adrenal cortex in thyrotoxic children. In adults there is a relative adrenal insufficiency in thyrotoxicosis, and the same situation may exist in children who have had the disease for a long period.

Gonads

Sex distribution is similar to that in the adult form of the disease. Hyperthyroidism in children primarily affects females (30). In the present series 48 of 50 patients were females (Table 2)—a ratio of 24 females to 1 male. Sattler (2) observed that with the approach of puberty there was an increase in the percentage of girls affected. In the age group 0–5 years, he found 37 per cent boys; in the 6–10 age group, 25 per cent boys; and in the 11–15 age group, 20 per cent boys. In Bram's (5) series of 128 cases, the ratio was 16 females to 1 male; in Kerley's series (31), 3 females to 1 male; and in Kennedy's series (4), 7 females to 1 male. The latter ratio agrees with that observed by Talbot (19). The preponderance of female children with this condition and the increased incidence at the time of prepuberty or puberty strongly suggest some disturbance in the pituitary-thyroid-gonad axis.

Relationship to puberty. There are many diverse opinions regarding the effect of hyperthyroidism on the processes of sexual development. Reilly (20) contends that thyrotoxicosis suppresses ovarian function. Lehman (32) holds that physical overdevelopment as well as precocious sexual development is not uncommon in hyperthyroidism. Precocity was observed in only 1 child in our series.

Alterations in the menstrual pattern may be of different types. Holmgren in 1909 (24) observed that in 73 per cent of cases menses started at 15 years of age or under; in 1 case the onset was at 9 years; in 27 per cent the onset was between 16 and 22 years. Atkinson (1) noted that the onset of menstruation was often delayed, and Piper (33) stated that puberty was delayed by from two to four years. Welti (34) reported that menstruation started late or became irregular and scant, even to the point of amenorrhea. Reilly (20) noted that secondary sex characteristics appeared slowly in a group of hyperthyroid children; menstruation did not occur in 4 children

and was scanty in 4 others; in 9 menstruating children, amenorrhea developed.

Our series provided an excellent opportunity to re-evaluate this particular feature. Adequate menstrual histories were available in 36 of the 41 female patients at the age of puberty or beyond. In Figure 3 is a summary of this information with a separation of cases into those whose menses developed prior to thyrotoxicosis and those in whom the onset followed the thyrotoxicosis. In neither group was there any striking effect of the

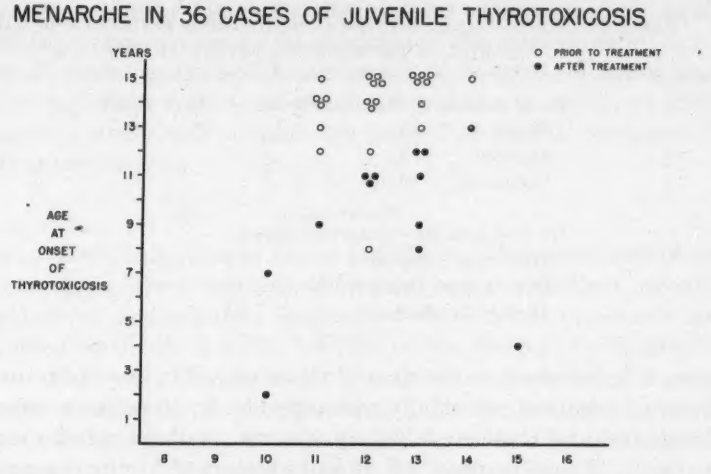

FIG. 3. Age at menarche (abscissa) compared to age at onset of hyperthyroidism (ordinate).

menstrual pattern. In 31 of the 36 patients the onset of menses was between the ages of 11 and 13 years. In no patient was there a marked delay in the onset of puberty; in 1 patient it was at 15 years of age, which is not unusual in any group of normal individuals. Two patients had their first menses at age 10; this may also occur among normal girls. Therefore, from these data there is no evidence that juvenile thyrotoxicosis seriously alters menstrual patterns. The delay in onset which is often mentioned by other authors appears frequently to have been based on statistically insufficient data. Talbot (19) stated that there may be a delay in onset of menses if hyperthyroidism precedes the menarche, and a tendency to oligomenorrhea or amenorrhea if hyperthyroidism follows the menarche. However, he does not mention any figures regarding the frequency of these findings. Talbot also obtained a history of abnormal menses in about 30 per cent of 38 patients with thyrotoxicosis who had reached the menarche. Our inci-

dence is distinctly different in that only 8 per cent of 36 patients had any abnormality of menses.

The effects of thyrotoxicosis on puberty are not limited to female children. Welti (34) observed 2 boys with small testes and no pubic hair. In 1 of these an undescended testis descended after thyroidectomy. Reilly (20) also observed retarded sexual maturation in 1 boy with hyperthyroidism.

As a further exploration of the effect of pubertal or postpubertal thyrotoxicosis on gonadal function the marital and obstetrical histories were obtained in 19 cases (Table 10). Forty-two of the 49 living patients (in-

TABLE 10. MARITAL AND OBSTETRICAL HISTORIES OF PATIENTS WHO HAD PUBERTAL OR POSTPUBERTAL THYROTOXICOSIS

No. of patients of marriageable age		42 (1 male)
Single	13	
Married	19	
Unknown	10	
Married patients—obstetrical history		
History of pregnancy	19	
One or more living children	17	
History of abortion	5	

cluding 1 male) were, at the time of this study, 20 years old or over, and therefore considered potentially marriageable. In 10 patients information as to marital and obstetrical history was not available; of the remaining 32 patients, 19 were married. All 19 had a history of 1 or more pregnancies; and 17 had given birth to at least 1 living child. Five patients had a history of abortion. These findings indicate that there is no long-term effect of juvenile thyrotoxicosis on the ability to conceive at a later age.

In summary, we are of the opinion that thyrotoxicosis does not significantly alter the age of onset of menses. Alteration in menstrual pattern occurs in very few patients. The best long-term evaluation of the effect of juvenile thyrotoxicosis on ovarian function is obtained from a study of pregnancies when these patients reach marriageable age. In this series, all married females had at least 1 pregnancy, and in none was there a history of infertility or sterility.

Pancreas

Diabetes has its greatest incidence during the periods of the menarche and the menopause. Therefore it is reasonable to wonder what effect, if any, juvenile thyrotoxicosis has on the development of this condition. In only 1 patient did diabetes mellitus develop. The diagnosis of thyrotoxicosis was made at the age of 11; menarche occurred at age 12; and diabetes

developed at age 27. This patient is now 33 years of age. Although the series is small, and not all of the patients have been followed long enough in their life span to state definitely the incidence of diabetes, it appears thus far that the incidence will not exceed the normal probability.

It has been of particular interest to note the relative infrequency of endocrine alterations as either precursors or sequelae of juvenile thyrotoxicosis. Previous concern about possible disturbances in growth, alterations in menses and fertility, and other endocrine disturbances, appears to have been overemphasized. The most important determinants as to whether endocrine or metabolic disturbances will occur as a result of juvenile thyrotoxicosis are 1) the duration of the disease before it is recognized, 2) its intensity, and 3) the length of delay in initiating therapy which is adequate to restore a state of euthyroidism as rapidly as possible. This applies equally well to either the surgical or medical management of juvenile thyrotoxicosis.

DISCUSSION

Prior to the introduction of iodine in 1923 as a therapeutic measure for hyperthyroidism, the treatment of this disease in children essentially consisted of rest and sedation. Surgical interference was hazardous, and it was avoided whenever possible because of the danger of complications. Moreover, the cyclical nature of the disease plus the belief that spontaneous arrest would occur, also rendered justifiable the prolonged hospitalization, the restricted activity and the attendant psychic trauma of medical management.

The beneficial therapeutic effects of iodine have been observed in the control of moderate thyrotoxicosis in these younger subjects, but of far greater value has been its usefulness in establishing a euthyroid state in preparation for definitive surgical treatment. The reduction of operative risk directly attributable to iodine is reflected in Kennedy's (3) report from the Mayo Clinic: 5 deaths occurred prior to 1922 among 55 surgically treated children; 4 deaths occurred between 1922 and 1939 among 91 similarly treated. However, the results of operative treatment in the iodine era were assessed as good in 68 per cent of the cases as opposed to 32 per cent prior to 1922.

The introduction of potent goitrogenic derivatives of thiourea in 1943 (35) heralded yet another era in the problem of thyrotoxicosis in childhood. Despite the not infrequent complications of the prolonged use of these drugs, most cases can be controlled while the drug is being administered. Early inexperience with these compounds resulted in a few cases of myxedema—a complication rarely encountered today. The question of the carcinogenic properties of these potent goitrogens, especially when

used in the young, has been reviewed by Cope (36). As yet there is little substantiation for this effect.

The initial enthusiasm with which thiourea derivatives were received and employed was justified by certain experiences with the surgical treatment of childhood thyrotoxicosis. Many reports, notably those of Kennedy (3), Reilly (20), Topper (37, 38), Finkler and Becker (39), Kunstadter and Stein (40, 41) and Drake *et al.* (42), indicated an alarming frequency of postoperative myxedema. Certain of these authors (Topper, and Kunstadter and Stein) presented cases of disturbed growth or maturation, despite the fact that the surgically induced hypothyroidism had been treated with an exogenous source of thyroid hormone.

We feel that our experience has affirmatively answered Topper's question regarding the propriety of thyroidectomy in children: "Will the child grow and mature normally? Will the remaining thyroid tissue be sufficient to meet the future needs of the growing organism?"

Other complications of thyroid surgery also became preventable with the introduction of thiourea compounds. The infrequent but always potential postoperative death, and the alleged frequent postoperative thyroid crisis and hypoparathyroidism were no longer feared; nor was there the former concern about recurrent laryngeal nerve injury and keloid formation, both reportedly (Topper (37, 38); Kunstadter and Stein (40, 41)) more frequent in children than adults.

In the cases reported here, thyroidectomy after suitable preparation has consistently resulted in complete, rapid and permanent control of thyrotoxicosis, with few complications. Growth disturbances following thyroidectomy are not common, as once believed, provided care is exercised in maintaining a normal thyroid state postoperatively.

Reports of remission of thyrotoxicosis during treatment with thiouracil, propylthiouracil and methylthiouracil were collected from the literature by Topper in 1948 (37), and she added her own experience. Jackson and Haley (43) treated 8 cases with these urea compounds; in 1 there was permanent remission after thirteen months' administration of propylthiouracil, and in 4 remission was maintained only during the administration of the drug. Kunstadter and Stein (41) added 9 cases; in 5 (4 moderately thyrotoxic; 1 severe) permanent remission resulted from drug therapy. Four other subjects of these authors (all "severely" thyrotoxic) underwent thyroidectomy because of relapses during therapy, a goitrogenic effect, or inability to administer the drug. Drake *et al.* (42) reported 2 patients rendered euthyroid by propylthiouracil; in neither case had the drug been withdrawn. Topper (38) observed a sustained remission with propylthiouracil in 1 case in 1951. Piper's (33) case suffered a relapse three weeks after cessation of a regimen of rest and sedation, but the hyperthyroidism subsequently was under control with methylthiouracil.

In all but a few of the reported cases, the reversal of hyperthyroidism was attained only during the necessarily prolonged period of administration of the thiourea derivatives. Continuous administration of these drugs was precluded because of the possibility of certain effects, notably enlargement of the thyroid gland and leukopenia. Jackson and Haley (43) made special mention of the uncertainty of metabolic response to goitrogens; Lyons (9) notes the high relapse rate in adults after cessation of administration and saw no reason to believe it would be lower in children.

In our opinion, antithyroid compounds have a most important role in the management of thyrotoxicosis. When employed in the treatment of juvenile hyperthyroidism, full adult doses of the drug should be administered, and should be continued long enough to restore a euthyroid state, maintained for twelve to eighteen months. Factors leading to a decision to undertake surgical resection are: 1) failure of the drug due to toxicity, 2) a relapse after a medically induced remission, 3) progressive enlargement of the goiter under medical treatment, 4) psychic factors in the parent or child that make adequate observation during prolonged therapy doubtful, 5) economic problems of the family, and 6) persistence of mild hyperthyroidism while the patient is receiving an adequate dose of the drug. A medical remission must secure permanent relief from the symptoms and leave the patient with no visible or obstructively large goiter.

The ease and safety with which thyroidectomy can be performed after proper preparation with antithyroid drugs has already decreased the incidence of sequelae. Being able to take the time to carry out a careful anatomic dissection and to preserve the recurrent laryngeal nerves and parathyroid bodies is an enormous advantage enjoyed by surgeons today. The fact that adequate replacement therapies are available for the control of postoperative hypothyroidism and hypoparathyroidism does not lessen the surgeon's desire to avoid these hazards. Experience is a major factor in their prevention, but even when it is known that parathyroid tissue has been left in situ, tetany may ensue.

Prior to the advent of goitrogens, Reilly (20) in 1940 made the following statement which is still worth remembering: "In the treatment of extremely severe exophthalmic goiter in children, surgical intervention has offered the most aid for arresting toxicity. Other methods often only temporize; however, they may help to prepare for safer and more curative surgery. Nevertheless, surgical intervention may be only palliative for some children, since it does not always remove the cause of such overactivity of the thyroid gland."

CONCLUSIONS

Although hyperthyroidism in children is a rare condition, it occurs with sufficient frequency to make it a problem of importance. There is suggestive evidence that its incidence is increasing.

The longer hyperthyroidism is permitted to exist in children the more deleterious are its effects. If it has not existed over a long period, and if it is properly controlled, it does not alter the normal physical and endocrine development. The emphasis in the literature on menstrual disturbances caused by this disease is not justifiable. In our series normal fertility was observed in all the patients who were followed after marriage.

Early, adequate control of juvenile thyrotoxicosis is essential. A medical regimen may be successful, but surgical resection of the thyroid gland remains the most satisfactory method of therapy. Antithyroid drugs should be given in full adult doses and the patient thus maintained in the euthyroid state for twelve to eighteen months, unless indications for surgery arise.

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METABOLITES OF HYDROCORTISONE AND CORTISONE IN SYNOVIAL FLUID IN RHEUMATOID ARTHRITIS*

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INTRODUCTION

IT HAS been demonstrated that several tissues have the capacity to effect chemical transformations of added steroid hormones *in vitro*. Two recent reviews (1, 2) summarize much of the work pointing to the liver as a major site of catabolic changes, and to the kidney as also active in this respect. Other tissues appear to act on specific steroid compounds (3–8). When endocrine tissues transform added steroids to the characteristic active hormones of these tissues (6, 8) the process may be classed as biosynthetic rather than catabolic. The biosynthesis of adrenocortical hormones from inactive precursors by adrenal tissue has been amply demonstrated by the Worcester group and others (1, 9).

Because of its accessibility, the synovial cavity offers an excellent opportunity for observations on the *in vivo* metabolism of steroid substances by a peripheral tissue in health and disease. The studies here reported confirm our previous finding that rheumatoid synovial tissue can metabolize the adrenocortical hormones, cortisone and hydrocortisone (10). In preliminary *in vitro* incubation experiments we found that this is not exclusively a property of inflamed tissue, since both normal and rheumatoid synovia were capable of producing metabolites from added hydrocortisone.

The possible relationship between the physiologic activity of the active agent and its chemical transformation is also of interest. It is well known that cortisone and hydrocortisone do not differ greatly in anti-inflamma-

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tory activity when administered systemically (11). Upon being injected directly into the synovial cavity, however, cortisone has little or no effect, whereas hydrocortisone is strongly anti-inflammatory (12, 13). Since these two compounds have closely similar activities in other respects, this unique difference suggests that chemical transformation is involved in the anti-inflammatory effect. It was felt, therefore, that a comparative study of the metabolites produced from cortisone and hydrocortisone might throw light on the mechanism by which their anti-inflammatory activities are exerted.

We have also investigated the possibility that some of the steroids which were injected, or their metabolites, might be rendered non-extractable from synovial fluid as a result of conjugation, or of binding to the proteins. Aspirated fluids, after extraction with chloroform, were re-extracted after incubation with β-glucuronidase and subsequently after incubation with papain. Additional steroid material was obtained as a result of these procedures.

It has been shown (10) that cortisone, hydrocortisone and their acetates disappear rapidly from the synovial fluid after intra-articular injection. Hence, the metabolites available for study were only those present in the small amount of steroid material which remained at the time the fluid was aspirated.

MATERIALS AND METHODS

Subjects: All subjects had rheumatoid arthritis with effusions in one or both knees. All were receiving oral maintenance therapy with cortisone acetate, administered daily in four doses totalling 75 or 100 mg.

Intra-articular injection and collection: The techniques for injection of the steroid, and aspiration and extraction of the synovial fluid with solvent, have been previously described (10). In the present study, each injection experiment was performed four times in the same patient, allowing an interval of at least two weeks between injections. After the fluid was aspirated from the synovial cavity of the knee joint, it was extracted with chloroform; the four extracts collected in each experiment were combined. Thus the compounds remaining in the synovial fluid after the injection of 400 mg. of steroid were available for chromatographic analysis.

In 3 experiments, analyses were made of fluid collected:

A. Half an hour after the injection of free hydrocortisone alcohol.

B. Three hours after the injection of free hydrocortisone alcohol.

C. Three hours after the injection of free cortisone alcohol.

Procedural details of the analytical techniques are given in subsequent Experimental sections.

Controls: In Experiment A, only subjects with bilateral effusions were included. Steroid was injected into only one knee joint, the other serving as a control; the contents were aspirated from each joint simultaneously. In Experiments B and C, knee-joint fluids were collected before the injection of steroid, either by partial aspiration of the joint which was to receive the injection, or by aspiration of the contralateral joint.

Enzyme treatment of extracted synovial fluid: In Experiment A, after chloroform extraction, the fluids (including the controls) were treated first with β-glucuronidase and then

with papain. A volume of water equal to that of the combined control synovial fluids was treated in the same manner. The mixtures were re-extracted with chloroform after each enzyme treatment, and the extracts were analyzed in the same manner as the first extract made before enzyme treatment.

Quantitative assays before chromatography: The crude neutral extracts were assayed for corticosteroids by the following procedures:

1. Formaldehydogenic substances were determined by a direct procedure employing ether extraction of the reaction mixture after oxidation (14).
2. Reducing substances were estimated by a semi-micro adaptation of the acid molybdate procedure of Heard and Sobel (15).
3. Steroids reacting with phenylhydrazine were determined by a semi-micro modification of the Porter-Silber reaction (PS) (16), carried out at room temperature (17).

Paper chromatography: The extracts were chromatographed using the toluene-propylene glycol system of Burton and Zaffaroni (18, 19). Details of the method are given in the Experimental section at the end of this article. In general, chromatograms were first developed for seven days ("Slow" paper). The run-off was collected, evaporated, and reapplied to another paper which was developed for two days ("Medium" paper). This run-off was reapplied for five hours ("Fast" paper). In some instances the five-hour run-off was again applied, using the ligroin-propylene glycol system of Savard (20).

After drying, the papers were examined under a short wavelength ultraviolet lamp, which shows the Δ^4-3-ketones as dark purple bands. Test strips, 2 mm. wide, were cut off and tested in turn with triphenyltetrazolium chloride, which shows reducing corticosteroids; 2,4-dinitrophenylhydrazine (19), which also reveals Δ^4-3-ketones; and fuming sulfuric acid, which forms fluorescent areas with some steroids under ultraviolet light.

Cutting and elution of chromatograms: The papers were cut so as to separate regions reacting in the foregoing tests, and each cut was eluted with absolute ethanol. Special precautions were used in order to minimize contaminants (*see* Experimental section). Eluates were dried, re-dissolved in chloroform, and washed with water.

Quantitative assays after chromatography and elution: Eluates were assayed by the application of a sequence of reactions, in the following manner:

1. A determination of total 17-hydroxysteroids and 17-ketosteroids, by means of a technique employing chromic-acid oxidation followed by a micro Zimmermann reaction (CrO_3-KS) (21, 22).
2. When the CrO_3-KS reaction was positive, the eluate was further assayed for 17-hydroxy-α-glycols (HIO_4-KS) (23).
3. The content of preformed 17-ketosteroids (17-KS) was determined in conjunction with the HIO_4-KS assay (23).
4. Eluates which reacted positively in the CrO_3-KS assay were also tested by the Porter-Silber (PS) reaction (17).
5. Eluates which reacted negatively in the CrO_3-KS determination were not further assayed. However, most eluates were tested for absorption in concentrated sulfuric acid (SAC) (24). This procedure reveals the presence of steroid material which is not reactive in the CrO_3-KS determination, as well as yielding information as to its purity.

Formaldehydogenic assays on material eluted from papers impregnated with propylene glycol could not be relied upon; for even though the washing procedure presumably removed most of the propylene glycol, the values obtained were often well above the other indices of steroid concentration. Molybdate reduction assays were not performed on eluates; reducing power was judged by the triphenyltetrazolium chloride spot tests on the papergrams.

RESULTS

Absorption of steroids from synovial fluid after injection: We have previously noted that when assays for corticosteroids were employed, both cortisone and hydrocortisone appeared to be rapidly removed from the synovial fluid. The average recovery after one-half hour was 21 per cent (Experiment A) and after three hours it was about 2 per cent (Experiments B and C). Detailed analyses of these extracts have been given (10). Subsequent assays after chromatography and elution revealed additional material which reacted in either the CrO_3–KS procedure or with H_2SO_4.

These compounds would increase the amount of steroid remaining three hours after injection to a maximum of about 8 per cent.

Pre-injection control fluids: An analysis of pre-injection synovial fluid (Experiment B) furnished no conclusive evidence of the presence of steroid compounds. No definite reducing bands were seen on a 1-cm. wide chromatogram of this extract (Fig. 3). None of the eluates showed a positive CrO_3-KS reaction. The SAC spectra showed no peaks other than those due to the procedure control, with the possible exception of eluates BO1 and BO7. This finding is in accord with those of our previous experiments (10), in which only faint reducing bands were found on chromatograms of extracts of as much as 130 ml. of pre-injection synovial fluid.

Formation of metabolites after injection of steroids: The present experiments confirm and extend our previous findings, reported in preliminary form, that metabolites of the injected hormones appear in the synovial fluid (10). All chromatograms of post-injection fluids showed several bands in addition to that of the unchanged injected substance (Figs. 1, 3, 4). Since these materials were not found in pre-injection control fluids, they must have originated from the steroids which were injected.

Evidence that metabolites are formed in the synovial cavity: The possibility has been considered that the synovial tissue did not induce formation of the metabolites, but that they were formed elsewhere in the body (*e.g.*, the liver) from the steroid which had been absorbed from the knee. If the latter were the case one would expect to find equal amounts of metabolites in both knees after injection of only one knee. When such an experiment was performed, however, metabolites were much more abundant in the injected knee, as seen on comparison of the left-hand chromatograms of Figures 1 and 2. In spite of being spread on a chromatogram ten times as wide, the post-injection extract yielded more and stronger bands than did that from the contralateral knee. By CrO_3-KS assay, the post-injection metabolites (exclusive of unchanged hydrocortisone) totalled 8.6 mg. A total of only 0.9 mg. was found in the contralateral fluid; and of this amount the strong band assaying 0.5 mg. may have been unchanged hydrocortisone rather than a metabolite. Thus there is good evidence that the new compounds were formed in the synovial cavity either by the synovial tissue or by the inflammatory cells.

Evidence of the ability of synovial tissue to transform hydrocortisone has been afforded by preliminary *in vitro* experiments. Strips of freshly excised human synovial tissue were incubated in isotonic saline for three hours, with 10 mg. of added hydrocortisone. Spot tests on paper chromatograms showed two positive regions more polar than hydrocortisone, and two less polar bands. These were seen after incubating and extracting both normal and rheumatoid synovial tissue.

Patterns of Metabolites in Synovial Fluid

The following sections summarize the findings and conclusions from chromatographic analyses of post-injection and control fluids, both before and after enzyme treatment. Figures 1–4 show the chromatograms in schematic form, and Tables 1–4 give the results of quantitative assays on each eluate. Many of the fractions were further characterized by simple procedures which are adequate for the presumptive identification of

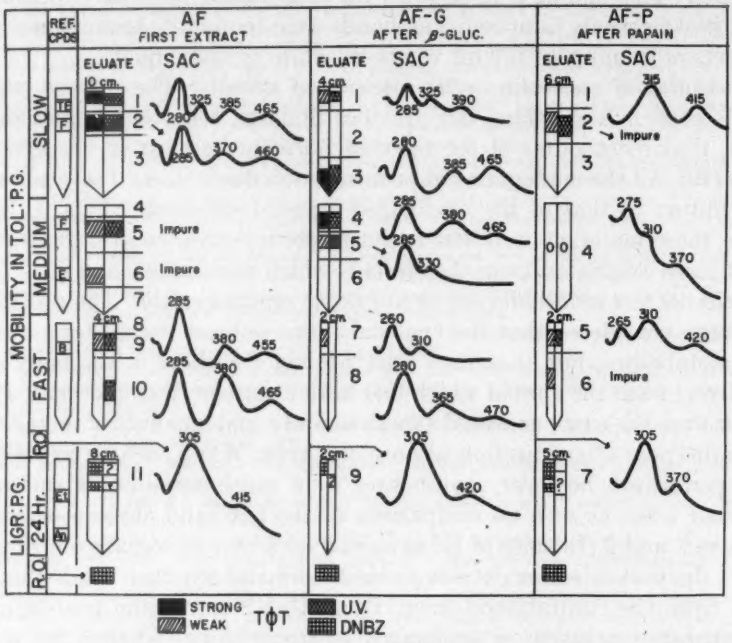

FIG. 1. Experiment A, chromatograms AF. Extracts of synovial fluid aspirated one-half hour after the intra-articular injection of hydrocortisone. Total amount injected, 400 mg.

SAC = absorption spectra of sulfuric-acid chromogens in mμ. The aliquots used for the test usually showed an absorption at 270–310 mμ approximately equal to that of 10 μg. of cortisone. Note: The contaminant revealed in procedure controls showed absorption peaks at 265 and 305 mμ; when present in sufficient concentration this resulted in displacement of peaks at 285 mμ. to a lower wavelength.

TE = tetrahydrocortisone; F = hydrocortisone; E = cortisone; S = 11-desoxy-17-hydroxy-corticosterone; B = corticosterone; A = 11-dehydro-corticosterone; Et. = etiocholane-3α-ol-17-one; An. = androsterone.

TϕT = reduction of alkaline triphenyltetrazolium chloride on the paper; U.V. = absorption band on the paper visible under ultraviolet light; DNBZ = positive reaction when sprayed with alkaline *m*-dinitrobenzene.

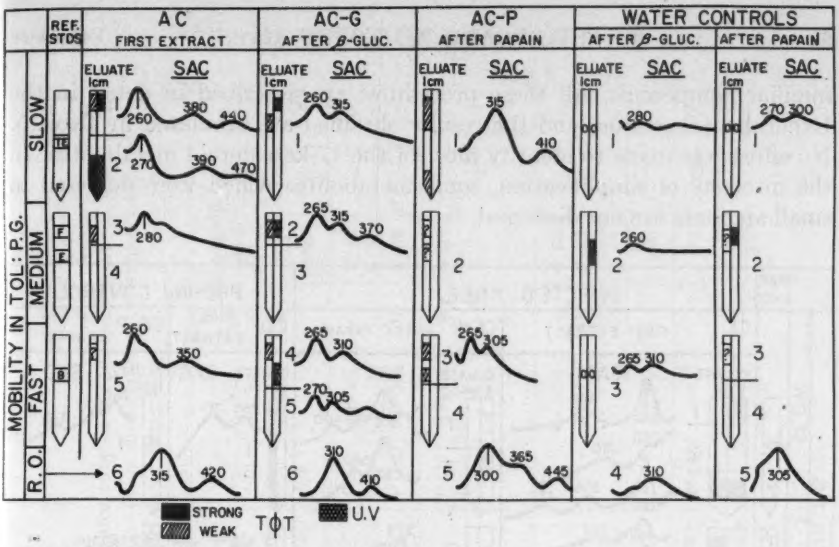

FIG. 2. Experiment A, chromatograms AC. Extracts of synovial fluid aspirated from the uninjected contralateral knee one-half hour after the injection of hydrocortisone into the opposite knee. Total amount injected, 400 mg.

The two right-hand chromatograms represent extracts of water which had been incubated with β-glucuronidase and with papain.

For explanation of terms, *see* Figure 1.

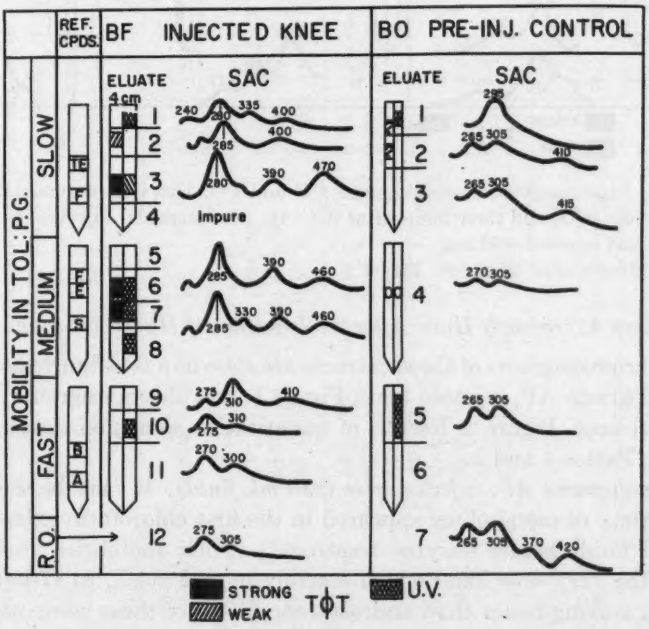

FIG. 3. Experiment B, chromatograms BF and BO. Extracts of synovial fluid aspirated before (BO) and three hours after (BF) the intra-articular injection of hydrocortisone. Total amount injected, 400 mg.

For explanation of terms, *see* Figure 1.

familiar compounds. All these procedures are described in detail in the Experimental section, and the results obtained are tabulated in Table 5. No effort was made to identify most of the 17-ketosteroid metabolites. In the interests of simplification, some metabolites which were detected in small amounts are not discussed.

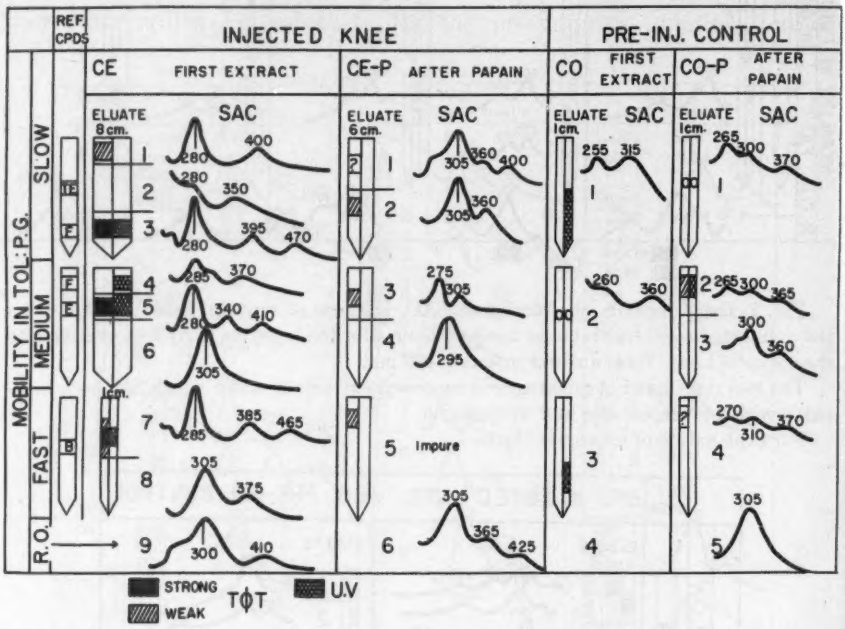

FIG. 4. Experiment C, chromatograms CE and CO. Extracts of synovial fluid aspirated before (CO) and three hours after (CE) the intra-articular injection of cortisone. Total amount injected, 400 mg.

For explanation of terms, *see* Figure 1.

Experiment A: One-half Hour After the Injection of Hydrocortisone

The chromatograms of these extracts are shown in two figures as follows: chromatograms AF, injected knee, Figure 1; and chromatograms AC, contralateral knee, Figure 2. Results of quantitative assays on the eluates are given in Tables 1 and 2.

Chromatograms AF, injected knee (230 ml. fluid): It may be seen that a wide variety of metabolites appeared in the first chloroform extract of the synovial fluid (before enzyme treatment). Their mobilities varied from that of the very slow band which scarcely moved at all, to 17-ketosteroid material moving faster than androsterone. Some of these compounds were

TABLE 1. EXPERIMENT A, CHROMATOGRAMS AF. ANALYSES OF ELUTED FRACTIONS FROM TOLUENE: PROPYLENE-GLYCOL PAPERGRAMS OF EXTRACTS OF SYNOVIAL FLUID ASPIRATED ONE-HALF HOUR AFTER THE INTRA-ARTICULAR INJECTION OF HYDROCORTISONE

(Total amount injected—400 mg.)*

Mobility in Tol.: P.G.	First extract					After β-glucuronidase					After papain				
	Eluate	TφT	CrO_3-KS (mg.)	17-KS (mg.)	PS (mg.)	Eluate	TφT	CrO_3-KS (mg.)	17-KS (mg.)	PS (mg.)	Eluate	TφT	CrO_3-KS (mg.)	17-KS (mg.)	PS (mg.)
SLOW	AF1	++	4.90	0	0.20	AF-G1	+	0.11		0.02	AF-P1	0	0.19		0
	AF2	+++	19.00†	0	19.50†	AF-G2	0	0		0.04	AF-P2	+	0.20	0	0
	AF3	0	0.23		0.20	AF-G3	+++	1.00†	0.25	0.75†	AF-P3	0	0.09		
MEDIUM	AF4	0	0.07		0.18	AF-G4	++	0.24†		0.05†	AF-P4	0	0.07		
	AF5	+	0.15		0.19	AF-G5	+	0.10		0.03					
	AF6	+	0.13		0.17	AF-G6	0	0.07							
	AF7	0	0.02		0.23										
FAST	AF8	0	0.06		0	AF-G7	+	0.06			AF-P5	+	0.03		
	AF9	+	0.34		0.15	AF-G8	0	0.14	0		AF-P6	+	0.12		
	AF10	0	1.28	1.34	0										
RUN-OFF	AF11		1.23	1.24		AF-G9		0.36	0.40		AF-P7		1.03	1.12	
Total			*27.41*	*2.58*	*20.82*			*2.08*	*0.65*	*0.89*			*1.73*	*1.21*	*0*

* Combined extracts from four injections of 100 mg. each. Total volume of fluids = 230 ml.

† In equivalents of hydrocortisone.

TABLE 2. EXPERIMENT A, CHROMATOGRAMS AC. ANALYSES OF ELUTED FRACTIONS FROM TOLUENE: PROPYLENE-GLYCOL PAPERGRAMS OF EXTRACTS OF SYNOVIAL FLUID ASPIRATED FROM THE UNINJECTED CONTRALATERAL KNEE ONE-HALF HOUR AFTER INJECTION OF HYDROCORTISONE INTO THE OPPOSITE KNEE*

Mobility in Tol.: P.G	First extract				After β-glucuronidase				After papain				Water control after papain†			
	Eluate	TϕT	CrO_3-KS (mg.)	17-KS (mg.)	Eluate	TϕT	CrO_3-KS (mg.)	17-KS (mg.)	Eluate	TϕT	CrO_3-KS (mg.)	17-KS (mg.)	Eluate	TϕT	CrO_3-KS (mg.)	17-KS (mg.)
SLOW	AC1	+	0.08	0	AC-G1	+	0		AC-P1	+	0.03		1	0	0.05	
	AC2	++	0.50‡													
MEDIUM	AC3	+	0.05‡		AC-G2	+	0		AC-P2	?	0		2	?	0	
	AC4	0			AC-G3	0	0									
FAST	AC5	?			AC-G4	+	0		AC-P3	+	0.02		3	?	0	
					AC-G5	0	0		AC-P4	0	0.03		4	0	0	
RUN-OFF	AC6		0.27	0.10	AC-G6		0.14	0	AC-P5		0.44	0.30	5		0.16	0.05
Total			*0.90*	*0.10*			*0.14*	*0*			*0.52*	*0.30*			*0.21*	*0.05*

* Combined extracts of 4 contralateral knee fluids. Total volume = 200 ml.

† Eluates of the water control after β-glucuronidase all showed a negative reaction in the CrO_3-KS assay.

‡ Estimated from intensity of TϕT reduction test on the paper.

revealed by the triphenyltetrazolium spot test on the papergrams (TϕT); others only by micro assay after elution.

Unchanged hydrocortisone was recovered to the extent of about 5 per cent, whereas the total titer of the metabolites by CrO_3-KS assay was about 2 per cent of the hydrocortisone injected. At least three cortico-

TABLE 3. EXPERIMENT B, CHROMATOGRAMS BF AND BO. ANALYSES ON ELUTED FRACTIONS FROM TOLUENE: PROPYLENE-GLYCOL PAPERGRAMS OF EXTRACTS OF SYNOVIAL FLUID ASPIRATED BEFORE (BO), AND THREE HOURS AFTER (BF) THE INTRA-ARTICULAR INJECTION OF HYDROCORTISONE

(Total amount injected—400 mg.)*

Mobility in Tol.: P.G.	BF, injected knee					BO, pre-injection control		
	Eluate	TϕT	CrO_3-KS	17-KS (mg.)	PS (mg.)	Eluate	TϕT	CrO_3-KS (mg.)
SLOW	BF1	0	0			BO1	?	0
	BF2	+	0.07	0	0.013	BO2	?	0
	BF3	+++	0.36†	0	0.40†	BO3	0	0
	BF4	0	0.10	0	0			
MEDIUM	BF5	0	0.07	0	0.09	BO4	0	0
	BF6	+++	0.35	?	0.31			
	BF7	+++	0.21	?	0.22			
	BF8	0	0.08	0	0.16			
FAST	BF9	0	0	0		BO5	0	0
	BF10	0	0	0		BO6	0	0
	BF11	0	0	0				
RUN-OFF	BF12		0	0		BO7		0
Total			*1.24*		*1.21*			

* Combined extracts from four injections of 100 mg. each. Total volume of post-injection fluids = 82 ml.; pre-injection control fluids = 69 ml.

† In equivalents of hydrocortisone.

steroid metabolites, of both greater and lesser polarity than hydrocortisone, and four 17-ketosteroids were found in the first extract. The corticosteroid metabolites were unknown compounds which could not be identified by the techniques at our disposal.

The very slow-moving fraction, AF1, is of particular interest, since its mobility could not be accounted for except by the introduction of new polar groups or configurations. The various tests and assays (Table 5)

TABLE 4. EXPERIMENT C, CHROMATOGRAMS CE AND CO. ANALYSES OF ELUTED FRACTIONS FROM TOLUENE: PROPYLENE-GLYCOL PAPERGRAMS OF EXTRACTS OF SYNOVIAL FLUID ASPIRATED BEFORE (CO), AND THREE HOURS AFTER (CE) THE INTRA-ARTICULAR INJECTION OF CORTISONE

(Total amount injected—400 mg.)*

Mobility in Tol.: P.G.	CE, injected knee; first extract					CE-P; after papain					CO, pre-injection control; first extract				CO-P; after papain			
	Eluate	TφT	CrO_3-KS (mg.)	17-KS (mg.)	PS (mg.)	Eluate	TφT	CrO_3-KS (mg.)	17-KS (mg.)	PS (mg.)	Eluate	TφT	CrO_3-KS (mg.)	17-KS (mg.)	Eluate	TφT	CrO_3-KS (mg.)	17-K (mg.)
SLOW	CE1	+	Lost			CE-P1	?	0	0	0	CO1	0	?+	0	CO-P1	0	0	0
	CE2	0	0.06	0	0.08	CE-P2	+	0	0	0								
	CE3	+++	0.76†	0	1.30†													
MEDIUM	CE4	0	0.10	0	0.03	CE-P3	+	0.21	0	0	CO2	0	0	0	CO-P2	+	0	0
	CE5	+++	0.42‡	0	0.30	CE-P4	0	0.08	0	0					CO-P3	0	0	0
	CE6	0	2.40	4.1	1.13													
FAST	CE7	++	0	0	0	CE-P5	+	0	0	0	CO3	0	0	0	CO-P4	0	0	0
	CE8	0	0	0	0													
RUN-OFF	CE9		0.30	0	0.02	CE-P6		3.68	2.46§	5.1	CO4		+	0	CO-P5		0.15	0‖
Total			*4.04*	*4.1*	*2.86*			*3.97*	*2.46*§	*5.1*							*0.15*	0‖

* Combined extracts from four injections of 100 mg. each. Total volume of post-injection fluids = 173 ml.; pre-injection control = 21 ml.
† In equivalents of hydrocortisone.
‡ In equivalents of cortisone.
§ Positive assay for 17-hydroxy-α-glycol = 2.22 mg.
‖ Positive assay for 17-hydroxy-α-glycol = 0.12 mg.

furnished evidence for the following structural features: An α-ketol was indicated by the reduction of TϕT on the paper, and a 17-hydroxyl group was shown by the formation of a 17-ketosteroid on oxidation with chromic acid. An almost negative reaction in the modified Porter-Silber procedure does not rule out a 17,21-dihydroxy-20-ketone, since some such compounds do not react (17). The Δ^4-3-ketone structure was shown by a characteristic absorption curve in ethanol, with a good peak at 240 mμ. Tentative evi-

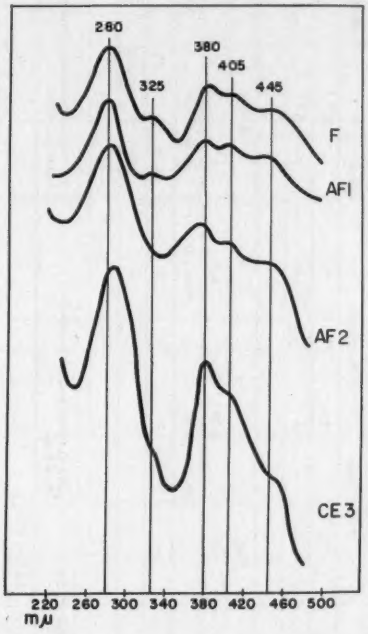

FIG. 5. Sulfuric-acid chromogen absorption spectra of the product of the oxidation of hydrocortisone with periodic acid, and of the oxidation products of various eluates (*cf.* Table 5).

dence for an 11-oxygen was found in the apparent formation of adrenosterone on chromic-acid oxidation, and the SAC after periodic-acid oxidation, which resembled the curve for oxidized hydrocortisone (Fig. 5). The very slow mobility of this substance cannot be accounted for by a 3-hydroxyl group, since it is a Δ^4-3-ketone. The slowest known $C_{21}O_5$ steroid with all the structural features we have found is 11 α-hydroxy-hydrocortisone (25). On the other hand, the actions of periodic and chromic acids, which yielded products apparently identical with those obtained from hydrocortisone suggest that the grouping which accounts for the high polarity

TABLE 5. CRITERIA OF CHARACTERIZATION APPLIED TO VARIOUS ELUATES

Eluate		AF1	AF2	AF6	AF9	AF10	AF-G1	AF-G3	AF-G4
Mobility of free cpd.		Slower than Tet.F	Same as F	Like E	Like B	Near A	Like AF1	Like F	Like F
Mobility of acetate		Slower than F acet.		Slower than E acetate					
Mobility of CrO_3 oxidation product		Same as adrenosterone							
Reduction of T⊕T on paper		+	+	+	+	0	+	+	+
Reaction in PS test		Almost negative	+	Slight	Slight	0	Almost negative	+	+
Oxidation to 17-ketone by CrO_3		+	+	?	+	+	+	+	+
Visible fluoresc. in conc. H_2SO_4		?	+	+	+	+		+	+
Ratio of titer by CrO_3-KS of acetate to other titers	CrO_3-KS of acetate	5							
	17-KS	High	High			0.9			
	PS	25	1.0†	0.25	2.3		5.5	1.33†	5.0
	Absorption of SAC*	0.8		0.25	Near 1.0	Near 1.0	Near 1.0	Near 1.0	Near 1.0
Evidence for Δ^4-3-ketone	U.V. on paper	+	+	0	+	+	+	+	+
	SAC peak at 285 mμ	+	+	?	+	+	+	+	+
	Absorption peak at 240 mμ	+				+			
SAC absorption spectrum*		Like 11α-OH-F+peak at 325 mμ	Like F	Like E, except no peak at 415 mμ	Like B	Same as Δ^4-androstene 11β-ol-3,17-dione	Resembles AF1	Very much like F	Very much like F
SAC after HIO_4 oxidation		Very like F product	Identical with F product	?					
SAC after CrO_3 oxidation		Like adrenosterone			Acetate did not give A acetate	Same as adrenosterone			
SAC of acetate		Same as free		Somewhat like free					
Conclusion or Identification		$C_{21}O_5$ or $C_{21}O_6$ 17-hydroxy-20,21-α-ketol Δ^4-3-ketone+other polar groups	F	$C_{21}O_4$ or $C_{21}O_5$ 20,21-α ketol, Saturated Ring A	$C_{21}O_4$ mixture with 17-hydroxy-20,21-α-ketol, Δ^4-3-ketone	Δ^4-Androstene-11β-ol-3,17-dione	Probably the same as AF1	F	F

* SAC is sulfuric-acid chromogen.
† Both titers calculated in equivalents of compound F.
? = Inconclusive result.
F = hydrocortisone; E = cortisone; Tet.F = tetrahydro compound F; Tet.E = tetrahydro compound E; S = 11-desoxy-17-dyhroxy-corticosterone; B = corticosterone; A = 11-dehydro-corticosterone.

TABLE 5—(*continued*)

Eluate		AC-2	BF1	BF2	BF6 & BF7	CE1	CE3	CE5	CE6	CE7
Mobility of free cpd.		NearF	Slower than Tet.F	Slower than Tet.E	Between E & S	Slower than Tet.E	Same as F	Same as E	Near S	Like B
Mobility of acetate		Near F acetate							Like a $C_{21}O_5$ diacetate	
Mobility of CrO_3 oxidation product										
Reduction of TφT on paper		+	0	weak	+	+(also+I_2-KI)	+	+(also+I_2-KI)	0	+
Reaction in PS test				+	+		+	+	sl. +	0
Oxidation to 17-ketone by CrO_3			?	+	+		+	+	sl. +	0
Visible fluoresc. in conc. H_2SO_4			+	+			+			+
Ratio of titer by CrO_3-KS of acetate to other titers	CrO_3-KS of acetate								3.0	
	17-KS						High	High	0.5	
	PS			5.0			0.6†	1.4	2.1	
	Absorption of SAC*			0.3			1.0	0.8	0.05	
Evidence for Δ^4-3-ketone	U.V. on paper	+	+	?	+	0	+	+	0	+
	SAC peak at 285 mμ	+		+	+	+		+	0	+
	Absorption peak at 240 mμ								0	
SAC absorption spectrum*		Very much like F	Somewhat like AF1	Somewhat like F	Somewhat like AF1	Unknown	Same as F	Like E	Single peak at 305 mμ	Like B
SAC after HIO_4 oxidation		Like F product		Unlike F product	Same as unox.		Same as F product			Somewhat like B product
SAC after CrO_3 oxidation		?			Like E				Single peak at 275 mμ	Acetate did not give A acetate
SAC of acetate		?							Same as free	
Conclusion or Identification		Probably F	May be same as AF1	17-OH-α-ketol. More polar than F	17-OH-α-ketol, Δ^4-3-ketone. More polar than S	Possible $C_{21}O_5$ steroid	F_e	E	$C_{21}O_5$ Saturated Ring A, 20-methyl or 20,21-α-glycol 3- or 20-ketone	$C_{21}O_5\Delta^4$-3-ketone 20,21-α-ketol

* SAC is sulfuric-acid chromogen.
† Both titers calculated in equivalent of compound F.
? = Inconclusive result.
F = hydrocortisone; E = cortisone; Tet.F = tetrahydro compound F; Tet.E = tetrahydro compound E; S = 11-desoxy-17-hydroxy-corticosterone; B = corticosterone; A = 11-dehydro-corticosterone.

may be associated with the side chain. An alternative explanation of the slow mobility would be that an additional oxygen group has been introduced, making a $C_{21}O_6$ steroid, though no direct evidence for the presence of a new oxygen function in the ring structure was obtained.

Eluate AF6, though directly opposite the cortisone standard, was found not to contain cortisone; and eluate AF9, though opposite the corticosterone standard, did not consist of corticosterone. Eluate AF9 was identified as Δ^4-androstene-11β-ol-3,17-dione (Table 5).

Eluate AF11, the run-off from the Fast paper, showed evidence of the presence of three more 17-ketosteroids when it was rechromatographed for twenty-four hours in the ligroin-propylene glycol system. The strongest band had scarcely moved at all, suggesting that it contained a $C_{19}O_3$ compound, retaining its 11-oxygen function (20). Fractions less polar than androsterone and etiocholanolone, reacting in the *m*-dinitrobenzene test (the 24-hour run-off), may have contained 17-ketosteroids which had lost their 11-oxygen group.

Following incubation with β-glucuronidase and re-extraction, further corticosteroids and 17-ketosteroids were obtained (chromatogram AF-G, Fig. 1; and Tables 1 and 5). New metabolites, not seen in the first extract, were AF-G7 (an unidentified corticosteroid) and AF-G8 (probably a 20-methyl-17-hydroxy steroid). The other materials, which appeared to be the same as those found before β-glucuronidase treatment, were unchanged hydrocortisone (1 mg.), a slow-moving substance like AF1, and at least two 17-ketosteroids.

Following incubation with papain and re-extraction, still further corticosteroid-like material was found, which was entirely different from any previously extracted (chromatogram AF-P, Fig. 1; and Table 1). The run-off showed a 17-KS titer almost as large as that of the first extract, amounting to 35 per cent of the total of all three extracts. Re-chromatography of the run-off in ligroin-propylene glycol yielded two bands reacting in the *m*-dinitrobenzene test in the same positions as those seen in the first extract; this run-off also gave a positive reaction.

Chromatograms AC, contralateral uninjected knee (200 ml. fluid): This extract, prepared from fluid withdrawn one-half hour after injection of hydrocortisone into the opposite knee, contained definitely more steroid compounds than did extracts of pre-injection control fluid (*cf.* Fig. 2, chromatogram AC; and Fig. 4, chromatogram CO). It is therefore probable that the steroid which was injected, and possibly its metabolites, diffused through the circulating body fluids and entered the contralateral synovial cavity. The chief constituent of the contralateral fluid was most probably unchanged hydrocortisone, in amount roughly 0.1 per cent of that injected (eluate AC2, Tables 2 and 5). A slow-moving band, eluate AC1, appeared to be the same as the corticosteroid eluate AF1 seen in fluid from the in-

jected knee, as judged by SAC before and after oxidation with periodic acid, and the reaction with chromic acid. Also in agreement with the findings on the fluid from the injected knee, was a substantial CrO_3-KS titer observed in the run-off both before and after enzyme treatment.

Comparison of the analyses of extracts from the injected and from the contralateral knee after enzyme treatment provides evidence that the additional material obtained from the post-injection fluid as the result of enzyme action originated largely from hydrocorticone, and was for the most part, not present before injection either in the synovial fluid or in the enzyme preparations. This is shown by the fact that the total eluate titers of extracts made after enzyme treatment were much higher for the fluid from the injected knee. When extracts of the latter, treated by β-glucuronidase, were assayed for CrO_3-KS, they contained fifteen times as much material as the corresponding extract from the uninjected knee; and the extract made after papain treatment, contained five times as much.

Figure 2 and Table 2 also show chromatograms of extracts made after water had been incubated, first with β-glucuronidase and then with papain. The only definite indication of steroid material was in the run-off after papain treatment, where a small CrO_3-KS titer was found, together with a peak at 305 mμ in the SAC. This titer was only about one-fourth that of the corresponding contralateral knee fraction, AC-P5.

Experiment B: Three Hours After the Injection of Hydrocortisone

The chromatograms of these extracts are shown in Figure 3 (chromatograms BF, injected knee, and chromatograms BO, pre-injection control, same knee). Results of quantitative assays on the eluates are shown in Table 3.

Chromatogram BF, injected knee (82 ml. fluid): Three hours after hydrocortisone injection there were fewer metabolites in the synovial fluid than after one-half hour, and the most abundant products were different. The slow-moving metabolite seen after one-half hour was no longer present in appreciable amounts, though traces of two substances more polar than hydrocortisone were detected (eluates BF1 and BF2, Table 5). The amount of unchanged hydrocortisone recovered had diminished to 0.4 mg. or 0.1 per cent of that injected.

The dominant metabolites were contained in a pair of bands of medium mobility, travelling between those for cortisone and Reichstein's substance S (eluates BF6 and BF7, Table 5). The total amount of these components exceeded that of the unchanged hydrocortisone. Although the SAC spectra of these bands were not identical, they may have contained the same component. They appeared to be different from those compounds found in the corresponding position in Experiment A. The SAC spectra of these bands were very much like those of the slowest-moving component,

and the band opposite corticosterone (eluates AF1 and AF9) in experiment A. Cortisone was not detected among the metabolites.

No faster-moving steroid material was detected by CrO_3-KS assay. Thus the 17-ketosteroids which were present after one-half hour had either been absorbed or further broken down.

Chromatogram BO, pre-injection control fluid (69 ml. fluid): Most eluates of this chromatogram had SAC curves which were dominated by the contaminant absorbing at 265 and 305 mμ. No definite evidence of the presence of steroid material was obtained.

Experiment C: Three Hours after the Injection of Cortisone

The chromatograms of these extracts are shown in Figure 4 (chromatograms CE, injected knee; and chromatograms CO, pre-injection control, same knee). Results of quantitative assays on the eluates are shown in Table 4.

Chromatogram CE, injected knee (173 ml. fluid): Three hours after cortisone injection the pattern of metabolites was quite different from that found after hydrocortisone.

Again, several metabolites of both greater and lesser polarity than the administered cortisone were found. The most polar band, CE1, was apparently different from the material of similar mobility seen one-half hour after injection of hydrocortisone. The band probably contained a mixture of substances, since the amount shown by absorption in sulfuric acid was more than the amount estimated from the TφT reduction on the paper. The clear-cut SAC spectrum indicated a Δ^4-3-ketone, but was not that of the 20α-hydroxy steroid corresponding to cortisone. Possibly a $C_{21}O_6$ steroid was present.

Noteworthy among the metabolites of cortisone in this experiment was hydrocortisone, which was conclusively identified in eluate CE3 by the criteria given in Table 5. The 1.3 mg. of hydrocortisone found by PS assay exceeded the 0.3 mg. of cortisone remaining unchanged after injection (eluate CE5; Table 5).

The most plentiful conversion product of cortisone was a non-reducing steroid which ran just ahead of the cortisone band (eluate CE6). The absorption of the SAC indicated the presence of 30 mg. of material—much more than was shown by any of the assays. The material, therefore, could be a 20-methyl steroid, or a 20,21-diol lacking both a 17-hydroxyl group and a Δ^4-3-ketone configuration (Table 5). The mobility of the acetate revealed the presence of two or more acylable hydroxyl groups, and a weakly positive Zimmermann reaction indicated either a 3-ketone or a 20-ketone, or both. This fraction was assumed to be a steroid in spite of the absence of conclusive evidence, because it was not found in the pre-injection control fluid.

Another metabolite of cortisone, found opposite the corticosterone standard, was shown to be a Δ^4-3-ketone with a 20,21-α-ketol side-chain, but no 17-hydroxyl group (CE7). The SAC of this band was of interest, since it was much the same as that of the band of similar mobility from experiment A, eluate AF9, though the evidence indicated that CE7 was a different substance. Both these SAC resembled that of corticosterone itself, but neither one contained corticosterone. Unknown corticosteroid metabolites were, therefore, again present. The run-off of this chromatogram (eluate CE9) also differed from the corresponding fraction three hours after hydrocortisone injection, in that it probably contained a 17-hydroxy steroid.

When the extracted synovial fluids were incubated with papain and then extracted a second time, additional material was obtained. The presence of steroid compounds in this extract was strongly indicated by their mobility on the papergrams, reduction tests on the paper, SAC absorption curves, and reaction in the CrO_3-KS assay (Fig. 4 and Table 4). The most polar fraction, CE-P1, was different from that of the corresponding eluate in chromatogram AF-P, as judged by its SAC absorption. This indicates that both fractions contained material derived from the respective hormonal injections. Eluate CE-P2 appeared to contain more of the same component, in purer form. Eluate CE-P5, the run-off of the five-hour paper, reacted in the 17-KS assay, and also in both the CrO_3-KS and HIO_4-KS tests. This is the only instance of a substantial titer of 17-hydroxy-α-gycols. Since the fastest-moving such compound we have seen is pregnane-3α,17α,20α-triol, which remains on the five-hour paper, the present reactant must be less polar, possibly a corresponding 3-ketone.

Chromatogram CO, pre-injection control (21 ml. fluid): No definite evidence of the presence of steroid compounds was obtained in the first extract of this fluid. After treatment with papain there was a small CrO_3-KS titer in the five-hour run-off (Table 4). Since this was also seen in the water control treated with papain (Table 2) it must be concluded that the enzyme preparation itself was the source of this material. The fact that papain liberated steroid material from the post-injection fluid but not from the pre-injection control again constitutes evidence that this procedure yielded additional metabolites of the hormone which was injected.

Bio-assays for Anti-inflammatory Activity

Three eluates were tested for local activity in inhibiting granuloma formation, by means of a modified rat granuloma-pouch method (26).[1]

[1] We are greatly indebted to Dr. Lewis H. Sarett of Merck and Company and Dr. C. A. Winter of the Merck Institute of Therapeutic Research for performing these assays.

Eluates AF1 and AF-P1 were inactive. Eluate BF7 caused inhibition of approximately the same degree as an equal amount of cortisone. Not only hydrocortisone, cortisone and corticosterone, but also a number of other 20,21-α-ketolic corticosteroids are known to be active in this test. Corticosterone is ruled out because of its much faster mobility; moreover the minor amounts of cortisone or hydrocortisone which could have been present would be insufficient to account for the activity. The possibility arises, therefore, that a new steroid, retaining activity in the granuloma test, has been produced intra-articularly from hydrocortisone.

DISCUSSION

It is evident that enzyme systems capable of effecting a variety of alterations in the steroid molecule are present in the synovial cavity. Specific reactions which have been found, or strongly suggested, include:

1. Reduction of the 4-5 double bond (AF6, AF11, AF-G8, CE6).
2. Reduction of 11-ketone to 11-hydroxyl groups (CE3).
3. Removal of 11-oxygen function (AF9, AF11-run-off, BF6, BF7 and CE7).
4. Removal of the tertiary 17-hydroxyl group (AF6, AF-P1, CE6).
5. Reduction of 20-ketone to 20-hydroxyl groups (CE-P5).
6. Reduction of 21-hydroxyl to 20-methyl groups (AF-G8).
7. Removal of the two carbon side chains (AF10, AF11).

Also suggested as possibilities are:

8. Introduction of new oxygen functions at positions other than 3, 11 or 17 (AF1, CE1).
9. Inversion of 11 β-hydroxyl compounds to 11α-epimers (AF1).

One of the latter two reactions offers a possible explanation for a Δ^4-3-keto-17,21-dihydroxy-20-ketone more polar than hydrocortisone (AF1). The metabolite does not seem to be identical with 6β-hydroxy-hydrocortisone (27), since our SAC curve is different. Inversion to an 11α-hydroxy epimer would presumably involve an 11-keto intermediate, but we did not detect cortisone in this experiment.

Comparison of the metabolites of hydrocortisone found at one-half hour with those remaining after three hours, provides evidence for a sequence of metabolic transformations occurring at different rates. For example, eluate AF1, at one-half hour, contained material which was formed more rapidly than it was broken down, so that it had accumulated at that time. After three hours this component had almost disappeared, but BF6 and BF7 had accumulated. The similarities in SAC spectra and other properties suggest that BF6 and BF7 may be derived from AF1.

A degree of selectivity can also be observed in the absorption of various compounds from the synovial cavity. Thus, three hours after injection of

hydrocortisone (Experiment B), the fast-moving metabolites had largely disappeared, but relatively large amounts of compounds of medium mobility remained, exceeding the amounts of compounds of similar mobility seen after one-half hour. In both Experiments B and C, the amounts of some metabolites exceeded that of the remainder of the steroid which was injected. Thus some substances were well absorbed, whereas others tended to accumulate in the synovial cavity.

In the present experiments no evidence for the presence of either tetrahydro compound E or tetrahydro compound F was found, nor has the presence of any 3-hydroxyl compound been proved. This is in contrast to the findings in urine, where these metabolites are regularly seen (28, 29). However, in a previous experiment (10) 1 subject apparently had converted cortisone acetate to tetrahydro compound E, rather than to hydrocortisone. Thus, there may be individual differences in metabolic pathways.

Comparison with unpublished chromatographic studies on urine extracts in our laboratory indicates that the intra-articular metabolites of cortisone and hydrocortisone retaining 21 carbons are also largely different from the other steroids in the urine of normal persons or of patients with untreated rheumatoid arthritis. Hence, the formation of abnormal metabolites by inflamed tissue cannot entirely account for differences in urinary corticosteroid excretion patterns between normal and rheumatoid subjects (30).

Nelson and Samuels (31), using the Porter-Silber reaction, found no arterial-venous drop in the titer of "17-hydroxycorticosteroids" across severely inflamed limbs, either before or after injection of ACTH, or intravenous infusion of cortisone and hydrocortisone. These authors conclude that there was little or no removal by peripheral tissues, making it difficult to maintain the theory of peripheral utilization of steroid hormones. If "utilization" is defined as the degradation of a steroid hormone to a less highly oxygenated or otherwise inactivated product, our experiments show that this does occur in synovial tissue. Similar extensive metabolism of steroid hormones may also take place in other hitherto unsuspected peripheral tissues.

It is obviously of interest to know whether the steroid which had left the knee was extensively altered on its way through the lining membrane, so that chiefly metabolites entered the blood, or whether the active hormone entered the blood largely unaltered. Our only information on this question comes from the materials which appeared in the fluid of the contralateral, uninjected knee one-half hour after hydrocortisone was injected into the other knee; the data indicate that some of the steroid found its way to the opposite knee unchanged (chromatogram AC, eluate AC2, Fig. 2, and Tables 2 and 5). If the chief component of this eluate is hydrocortisone, as

seems probable, it indicates that a large proportion of hydrocortisone entered the general circulation unchanged. Calculating from the amount of hydrocortisone which had left the fluid of the injected knee, the concentration of hydrocortisone in the fluid of the contralateral knee is that which would result from its distribution through 30 liters of body water. Other interpretations must, of course, be considered. Nevertheless, it seems possible that sufficiently large amounts of hydrocortisone can saturate the enzyme systems, not only of synovial tissue but of the liver and other tissues as well.

The unique difference between the anti-inflammatory activity of cortisone and that of hydrocortisone, following intra-articular injection, remains to be explained. The simplest explanation would be that cortisone must always be converted to hydrocortisone in order to develop anti-inflammatory activity, and that this reaction cannot be effected to a sufficient degree by synovial tissue. Indeed, it is known that cortisone can be converted to hydrocortisone in the body (32, 33), as well as by liver slices (34, 35). However, only a small proportion is so metabolized and numerous other metabolites of cortisone are found in urine, yet cortisone has the same magnitude of activity as hydrocortisone when administered systemically in rheumatoid arthritis (11). Thus it does not seem likely that cortisone acts through the formation of hydrocortisone.

An alternative hypothesis would be that both cortisone and hydrocortisone must be transformed into another product (or products) in order to exert their effects, and that synovial tissue can make this substance from hydrocortisone but not from cortisone. One form of evidence for this hypothesis is the fact that the observed metabolites of cortisone (which is locally inactive) were quite different from those of hydrocortisone. Had they formed the same metabolites, it would have argued against the possibility of activity in a metabolite.

It is especially interesting to find that cortisone can be converted to hydrocortisone in the synovial cavity, at least in some subjects. Why is cortisone, nevertheless, not active intra-articularly? Possibly not enough hydrocortisone was formed, yet the data of our experiment indicate that a substantial transformation had occurred. More hydrocortisone remained in the fluid than cortisone itself, though the latter was the steroid used for the injection. Moreover, the amount of new hydrocortisone formed from cortisone in Experiment C slightly exceeded the amount of hydrocortisone remaining after injection in Experiment B.

Another explanation could be that hydrocortisone itself is inactive, and that the anti-inflammatory effect is exerted by a metabolite to which it is converted in low yield, but which slowly accumulates in the joint. One particular metabolic fraction accumulated after injection of hydrocortisone

(BF6 and BF7), so that its total concentration at three hours was almost twice that of the remaining hydrocortisone. Hence, the fact that anti-inflammatory activity was demonstrated in a portion of this material is of particular interest.

The effect of β-glucuronidase in liberating additional amounts of steroid material for extraction must be due only in small part to hydrolysis of steroid glucuronides, since it is unlikely that the steroid which was injected had become extensively conjugated in this way. The additional hydrocortisone extracted after β-glucuronidase hydrolysis in Experiment A simply may have been that which remained from the first extraction. But the further yields of materials present in much smaller amounts, and of metabolites not seen in the first extract cannot be so explained. Possibly, our crude spleen preparation contained proteases or other enzymes which would account for these effects.

Papain was chosen for the second incubation because it is a proteolytic enzyme acting at pH 5.0, a point at which the corticosteroids are stable. The liberation of steroid compounds indicates that they were bound to the protein in synovial fluid by linkages which involved the intact protein structure and which were not available in peptides or amino acids. This form of binding must be different from that described by Eik-Nes *et al.* (36), in which the steroid remained freely extractable by solvent. The steroid compounds recovered fell into three groups: 1) hydrocortisone (injection), cortisone, and some metabolites not bound to protein in this way and extractable without papain hydrolysis, 2) other metabolites which were entirely bound, and 3) compounds which were partly free and partly bound. The largest fraction liberated by papain was of C_{19} structure. Of the total titer of preformed 17-KS in all three extracts in Experiment A, 13 per cent was obtained after β-glucuronidase and 35 per cent after papain hydrolysis. More polar steroids, to the extent of about 6 per cent of the total titer, were also liberated by each enzyme.

SUMMARY

1. Detailed chromatographic analyses have been made of synovial fluid aspirated from the knee joint half an hour and three hours after the intra-articular injection of 100 mg. of free hydrocortisone and of cortisone in subjects with rheumatoid arthritis.

2. Several metabolites were found, of both greater and lesser polarity than that of the steroid which was injected. These metabolites ranged from possible $C_{21}O_6$ corticosteroids to $C_{19}O_3$ and $C_{19}O_2$ 17-ketosteroids. Thus the ability of rheumatoid synovial tissue to metabolize hydrocortisone and cortisone *in vivo* has been confirmed.

3. Substantial amounts of hydrocortisone were identified after the in-

jection of cortisone. The patterns of metabolites of the two hormones were strikingly different. Since cortisone does not have anti-inflammatory activity in the knee, the local effectiveness of hydrocortisone may therefore involve the production of a specific metabolite.

4. When the extracted synovial fluid was incubated, first with β-glucuronidase and then with papain, and each incubation was followed by solvent extraction, further yields of steroids were obtained. Some of these steroids were the same as those in the first extract, but others were found to be liberated only after enzyme treatment.

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EXPERIMENTAL

Paper Chromatography

Whatman No. 1 paper was first washed in chromatogram tanks by running for one week in twice-distilled water and one week in 95 per cent ethanol. The paper was then dried in an oven at 40°C. ventilated by means of a fan. The finished chromatograms were similarly dried.

A 1-cm. width of paper was used per milligram of steroid material when the crude extracts were chromatographed. Regions of the resulting chromatograms showing strong evidence of corticosteroids were cut out and eluted. Regions showing weak or negative reactions were then combined, eluted, and rechromatographed on narrower papers. In this way, clearly defined bands could be obtained, even when the amounts of the various compounds differed widely.

Elution of papers and precautions to avoid contaminants

It was soon found that eluates prepared by simple procedures did not meet the requirements of the subsequent micro assays and sulfuric-acid chromogen (SAC) tests. A detailed study was therefore made of means of 1) reducing contaminants to a minimum, and 2) achieving complete elution of steroid compounds.

The most stubborn interfering contaminant was one which adhered to glassware even after being cleaned in chromic-concentrated sulfuric acid mixture. When concentrated sulfuric acid was allowed to stand for two hours in clean test tubes, its absorption spectrum showed definite peaks at 265 and 305 mμ, compared to the same acid placed directly into the spectrophotometer cuvette. This contaminant was also present in most solvents and could not be removed by re-distillation. Its absorption largely obscured the SAC curves when less than about 200 μg. of steroid was eluted from 5–10 sq. cm. of paper. Rigid adherence to the procedures described subsequently almost completely eliminated interference by contaminants. Extraneous formaldehydogenic substances eluted from the paper, as well as other undesirable materials, were removed by the washing procedure. Stills, evaporating outfits, and containers were all-glass. No rubber stoppers or tubings were used where they could possibly contaminate the extracts.

Cleaning of glassware

All glassware in any way related to the material being chromatographed was cleaned in a mixture of three parts of chromic-sulfuric acid plus one part of concentrated nitric acid. This included all solvent stills and containers, but did not include colorimeter tubes used in assays. The glassware was soaked in the cleaning mixture overnight, then rinsed ten times with tap water and five times with twice-distilled water. Once so cleaned, the glassware was not re-treated if it had been used only with solvents purified according to the following directions.

Purification of solvents

Chloroform: Wash twice with one-twentieth volume of concentrated sulfuric acid, or until no more yellow pigment is removed. Wash with redistilled water until the wash is

neutral. Dry the chloroform with anhydrous sodium sulfate (granular), filter through a fritted glass funnel, and distill, discarding the first and last 10 per cent. Store in the dark in a brown bottle at less than 15° C.

Ether: Place analytical reagent-grade ethyl ether (Mallinckrodt, in 5-lb. cans) in a separatory funnel and add about 100 ml. of cooled sulfuric acid diluted with water (50% v/v) per 5 pounds of ether. Shake, draw off the acid layer, and distill.

Absolute ethyl alcohol: Fill a large "column" with 1 pound of aluminum oxide (Merck, reagent grade) and allow 4 liters of absolute ethyl alcohol (U.S.I.) to percolate through it. Distill the filtered ethanol.

Toluene (or benzene): Dry over calcium chloride, then distill.

Ligroin: (Petroleum Ether A.R. (BP 30–60°, Mallinckrodt). Wash with sulfuric acid as for ethyl ether. Then wash with water, dry with anhydrous sodium sulfate, and distill.

Propylene glycol: Distill under high vacuum and store in the dark. Distill formamide also; then store in a desiccator over concentrated sulfuric acid.

Elution of paper chromatograms

After cutting the chromatograms, each section was again cut into narrow longitudinal strips. These were fed into the neck of a separatory funnel, and cut off at 0.5–1 cm. intervals. The cut papers in the funnel were then carefully wet with sufficient redistilled water to barely moisten them and absolute ethanol was added until the bits of paper were just covered. The funnel was closed, vigorously shaken for one minute, then allowed to stand and shaken twice more over a five-minute period. The funnel was swirled so that as much of the paper as possible adhered to the upper portions and the alcohol was allowed to drain out into an Erlenmeyer flask. This elution was repeated five times in all. The combined eluates were filtered through a fritted glass filter of medium porosity, then the paper pulp was transferred to the filter and washed with a little absolute ethanol, by means of suction. The ethanolic eluate was dried *in vacuo* under 40° C.

When cortisone (500 μg.) was applied to a paper previously impregnated with formamide, the recovery was 98 per cent as measured by the Porter-Silber assay.

Very small fractions (less than 150 μg.), such as those available after purification, were eluted with a minimum of solvent, in order to reduce contamination. The paper was cut into very small bits, transferred to a small test tube, and a few drops of ethanol added. After shaking and soaking, the ethanol was aspirated off into a vial. The elution process was repeated several times, and the combined eluates were then dried under a stream of nitrogen.

Washing of eluates

Large fractions: A procedure blank with solvent and water alone was carried through the following procedure with each group of eluates. The dried eluate was dissolved in 100 ml. of chloroform and washed through four separatory funnels containing 70 ml. of re-distilled water. The water washes were back-washed with two portions of 100 ml. of chloroform, which were combined with the main eluate. The chloroform was carefully evaporated in a vacuum to a small volume, and the fraction then transferred to a vial and dried with a stream of nitrogen.

This procedure removed formamide, or extraneous formaldehydogenic material derived from the paper, without loss of corticosteroid. When cortisone (200 μg.) was eluted and washed in this way, 95 per cent was recovered by formaldehydogenic assay.

Small fractions: Fractions of similar amounts were selected for each group, so that

the same volumes of the same batch of solvents could be used for all, and for the procedure blank. The dried eluate was taken up in 8 ml. of freshly washed chloroform per estimated 200 μg. of steroid material, and transferred to a small (30, 60 or 125 ml.) separatory funnel. After addition of 5 ml. of water to this funnel and to a second one, the chloroform solution was washed through both funnels. The water washes were backwashed once with 8 ml. of chloroform. The combined chloroform fractions were transferred to a round-bottomed flask with a ground-glass joint and evaporated in a vacuum by hand. For this process we have used an all-glass connecting tube, constructed to prevent refluxing of any solvent from the rubber tube leading to the vacuum line.[2]

The washing procedure for small amounts of material yielded good recoveries of cortisone eluted from paper, when judged by molybdate reduction or Porter-Silber assays. Formaldehydogenic titers were too high, indicating that some extraneous material remained.

Procedures for Characterization and Identification of Purified Fractions

Separation of mixtures

It was frequently found that regions of the chromatograms which showed well localized material by spot tests, nevertheless contained more than one compound. Two procedures were employed for separating such mixtures.

a) Re-chromatography: The eluted material was re-run on narrower or wider paper, as required, usually for a longer time.

b) Acetylation and re-chromatography of acetates: Approximately 0.17 ml. of dry pyridine and 0.10 ml. of acetic anhydride were added per mg. of dried material. After standing overnight at room temperature, the reagents were evaporated off under vacuum at 40° C.

The acetylated material was then re-chromatographed in such a manner that its mono-acetate would be expected near the middle of the paper, whereas if it formed a di- or polyacetate, this would be in the run-off. Compounds with only one acylable hydroxyl group could thus be separated from those with more than one such group.

Analytical chromatograms

Three strips of paper 0.5 cm. or 1.0 cm. wide, were left attached to the same head. To these were applied in turn, 1) a sample of the unknown, sufficient to give a positive reaction with the spot test, 2) a similar sample of the pure compound believed to be identical with the unknown, and 3) a mixture of these same amounts of each. If all three strips showed one positive band, the mobility of the unknown was considered to be the same as that of the pure compound.

Absorption spectra of sulfuric-acid chromogens (SAC)

Samples estimated to contain from 6 to 10 μg. of steroid were measured into 1.0-ml. tubes with ground rims, and dried overnight in a vacuum desiccator over P_2O_5. After adding 0.5 ml. of concentrated sulfuric acid the tubes were stoppered, inverted to dissolve the material, and allowed to stand two hours in the dark at room temperature. The reaction mixture was transferred to 0.5-ml. quartz cells, with a 1-cm. light path,[3] and read with a Beckman model DU quartz spectrophotometer, from 220 to 550 mμ. at 5 or 10 mμ. intervals.

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[2] Obtainable from E. Machlett & Co., 220 E., 23rd Street, New York, N. Y.

[3] Obtainable from Pyrocell Mfg. Co., 207 E. 84th Street, New York, N. Y.

The "elution procedure blank" for each set of samples was similarly run, and its absorption was subtracted from that of the eluted samples. In our experience this has been the most practicable correction to apply, since it was seldom possible to elute a "control" strip of paper, the previous treatment of which would be entirely comparable to that of an unknown.

Sulfuric-acid chromogens after oxidation with periodic acid or chromic acid

A "library" of expected SAC spectra was prepared by oxidizing the reactive compounds at hand, and by noting the published curves of presumed products (37, 38).[4] Comparison of the spectra of oxidized unknown samples has often given valuable information. Examples are given in Figure 5.

Oxidation with periodic acid

To a dried sample of the unknown, containing about 40 μg. of steroid, were added 0.10 ml. of absolute ethanol and 0.10 ml. of 0.01 M periodic acid in 0.15 M sulfuric acid. After one hour, 0.10 ml. of 6 per cent stannous chloride was added. The reaction mixture was then extracted once with 4 ml. of ethyl ether. The ether was washed four times with 2 ml. of water, dried with a little anhydrous sodium sulfate, decanted, and evaporated by means of a stream of nitrogen.

Oxidation with chromic acid

From 40 to 80 μg. of steroid material was thoroughly dried in a small test tube, and then dissolved in 0.04 ml. of glacial acetic acid (distilled from chromic acid). Chromic acid in glacial acetic acid, 0.06 ml. of an 0.2 per cent solution, was added and allowed to react for one hour at 40° C. The oxidation was then stopped by adding 0.05 ml. of ethanol. The reaction mixture was decanted into a small separatory funnel and extracted once with 4 ml. of ethyl ether. The ether was washed four times with 2 ml. of re-distilled water, dried with anhydrous sodium sulfate and evaporated with nitrogen.

Enzyme treatment of synovial fluid

β-Glucuronidase: To each ml. of diluted synovial fluid was added 100 units of β-glucuronidase from beef spleen. The mixture was adjusted to pH 5.0, and 1/50 volume of 1.0 N acetate buffer (pH 5.0) was added. After incubation at 38° C. for seventy-two hours, the mixture was extracted with chloroform as before.

Papain: The twice-extracted synovial fluid after treatment with β-glucuronidase was re-adjusted to pH 5.0 if necessary. Sodium cyanide, to a final concentration of 0.1 per cent of NaCN and papain[5] to a final concentration of 1.2 per cent, were then added. After incubating for forty-eight hours at 38° C., the mixture was again extracted three times with chloroform.

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[4] The authors are greatly indebted to the following persons for generous gifts of pure steroids: Drs. P. K. Frolich, R. Gaunt, G. Grant, W. J. Haines, F. E. Houghton, S. Lieberman, L. Sarett, J. J. Schneider, A. F. St. André, A. White and A. Zaffaroni.

[5] Obtainable from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

PROGESTERONE METABOLISM IN LIVER DISEASE

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IT HAS been established, chiefly by animal experimentation, that the liver is the site of conversion of progesterone to pregnanediol. The methods employed have been based on the lessening of progestational effect observed on implanting progesterone pellets into the spleen (1) or mesentery (2) or on the altered effects noted after partial hepatectomy (3, 4). Pregnanediol has been found in the bile following administration of Δ^5-pregnenol-3-β-one-20 to a postmenopausal woman (5) and following administration of progesterone in large amounts to patients with T-tube biliary drainage (6). Relatively few studies of progesterone metabolism have been reported in human subjects with hepatic disease (7, 8).

It is the purpose of this paper to present the results of measurement of pregnanediol excretion after a large oral dose of progesterone in a group of patients with various types of parenchymal liver disease and in a group with obstruction of the biliary tract.

CLINICAL MATERIAL

The subjects were 18 patients admitted to the New England Center Hospital with various types of liver disease. Six patients had some form of biliary obstruction, either extrahepatic or intrahepatic; the remaining 12 had parenchymal liver disease. The diagnosis was established in each instance by the history, physical examination and the usual liver function tests, including determination of the serum levels of bilirubin, total protein (plus albumin-globulin ratio), cholesterol and cholesterol esters, and measurements of cephalin flocculation and thymol turbidity. In a few patients bromsulfalein excretion was measured. In several instances a liver biopsy or laparotomy for the relief of biliary obstruction permitted direct confirmation of the diagnosis.

Each subject was given 500 mg. of crystalline progesterone contained in a capsule. Following administration of the progesterone, urine was col-

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lected in 24-hour aliquots for a period of five days, each specimen being quantitatively assayed for pregnanediol according to the method of Astwood, Grady and Wermer (9).

The control subjects were normal adults in whom there was no clinical evidence of hepatic disease.

RESULTS

Control group

In the control group of 10 normal adults the recovery of pregnanediol in the urine after an oral dose of progesterone ranged from 44.2 to 87.8 mg., the total recovery usually being about 10 per cent of the administered dose.

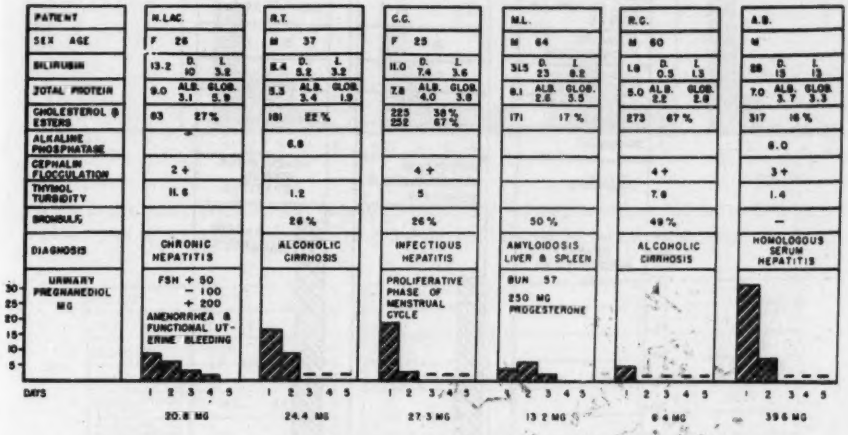

FIG. 1. Decreased pregnanediol excretion after oral administration of 500 mg. of progesterone, in 6 of 12 patients with parenchymal liver disease.

The major excretion occurred in the first two days and usually the excretion was less than 30 mg. the first day. These observations are in agreement with the results of other workers (10).

Parenchymal liver disease

The results in the 12 patients with parenchymal liver disease were variable. In 6 of the 12, the recovery of pregnanediol was well below that of the control group, and ranged from 6.4 mg. to 39.6 mg. (Fig. 1). Two of these 6 patients were women, and 1 of them (N. L., with chronic hepatitis) had amenorrhea alternating with episodes of functional uterine bleeding. Two of the 6 subjects had alcoholic cirrhosis; 2 had infectious hepatitis and 1 had amyloidosis of both liver and spleen. No consistent pattern of altered liver function distinguished these 6 patients from the others with paren-

chymal liver disease whose excretion of pregnanediol was either normal or increased.

In 2 of the 12 patients with parenchymal liver disease pregnanediol excretion was between 45 and 55 mg. in the first twenty-four hours (Fig. 2), an increase that was not observed in the control group. In 4 other patients (Fig. 3), the excretion was similar to that observed in control subjects. In

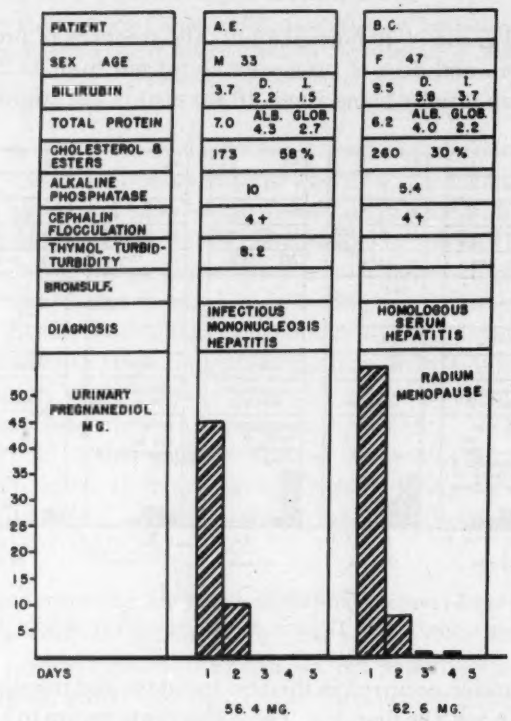

FIG. 2. Increased pregnanediol excretion on the first day after oral administration of 500 mg. of progesterone, in 2 of 12 patients with parenchymal liver disease.

1 of the female members of this group with chronic hepatitis, amenorrhea was present; the assay of follicle-stimulating hormone in the urine was negative for 5 units per day and there was a low estrogen index by vaginal smear. In another female patient sterility was a complaint but the menses were regular. In none of the 6 patients in the latter two groups was there a consistent clinical or laboratory abnormality to distinguish them from the 6 subjects in whom pregnanediol excretion was diminished.

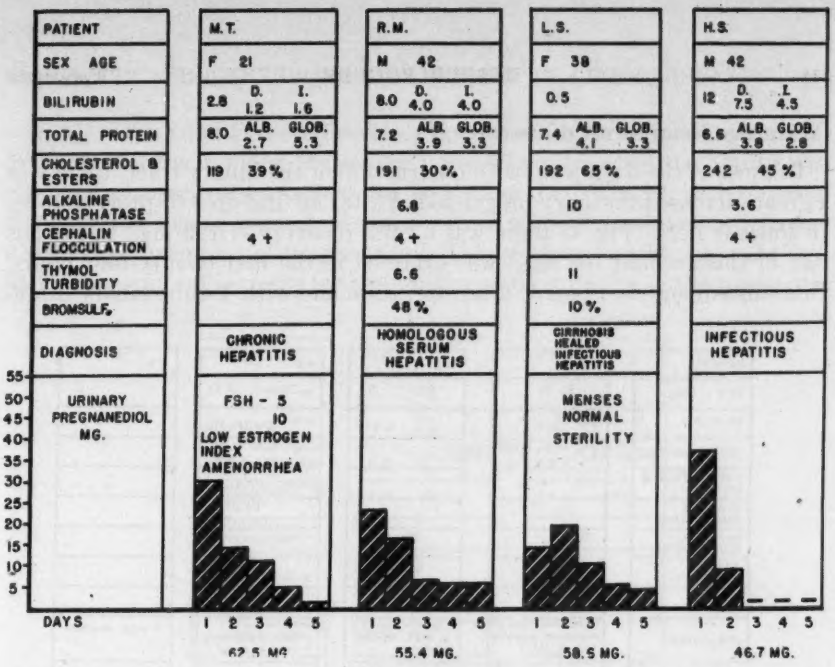

FIG. 3. Normal pregnanediol excretion pattern after oral administration of 500 mg. of progesterone, in 4 of 12 patients with parenchymal liver disease.

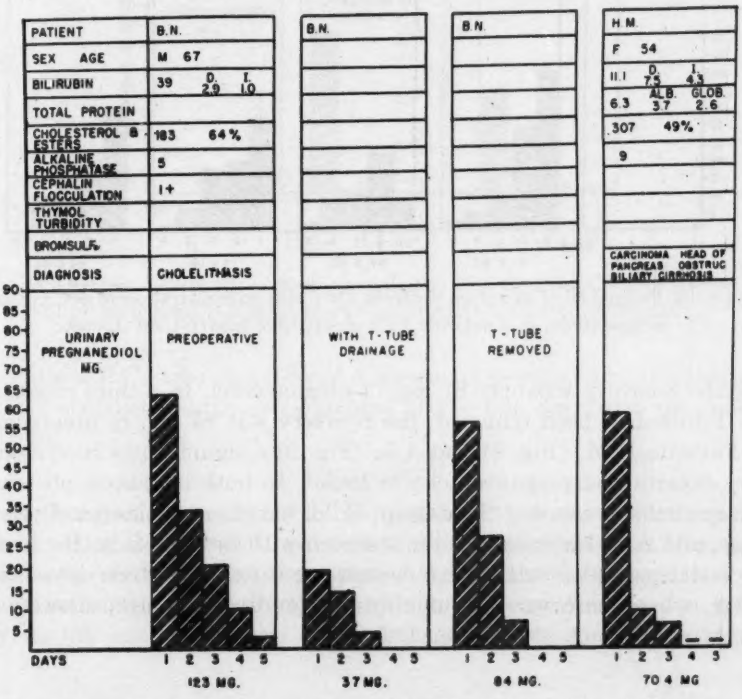

FIG. 4. Pregnanediol excretion patterns after oral administration of 500 mg. of progesterone in 2 patients with obstructive biliary-tract disease.

Obstructive biliary-tract disease

In most of the 6 subjects with obstruction of the biliary tract, there was a great increase in urinary pregnanediol after an oral dose of progesterone. In Patient B.N. (Fig. 4) there was a total recovery of 123 mg., but about half of this amount (62 mg.) was excreted in the first twenty-four hours. In a subsequent study made after operation and with T-tube biliary drain-

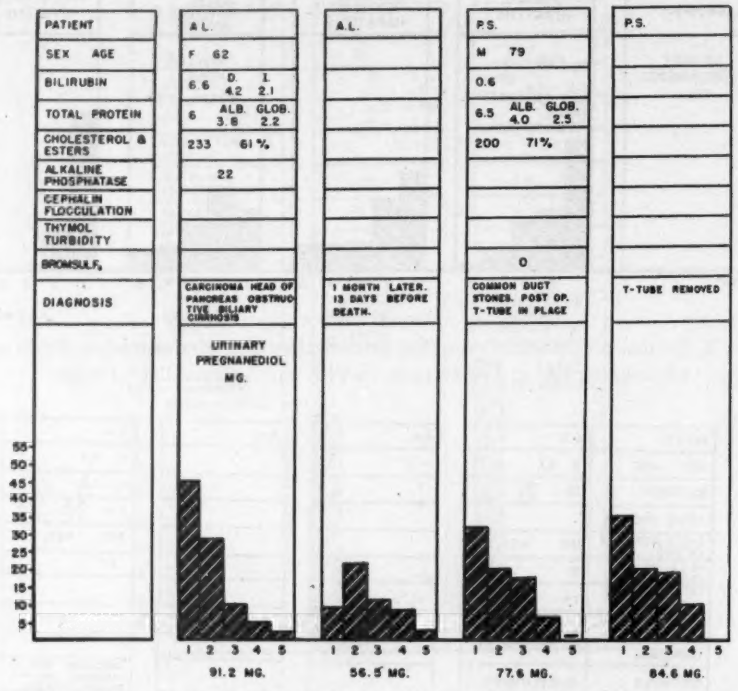

FIG. 5. Pregnanediol excretion patterns after oral administration of 500 mg. of progesterone, in 2 patients with obstructive biliary-tract disease.

age, the recovery was only 37 mg. of pregnanediol. In a third study after the T-tube had been removed, the recovery was 84 mg. of pregnanediol. In Patients H.M. (Fig. 4) and A.L. (Fig. 5) a significantly increased urinary excretion of pregnanediol was found. In both instances, obstructive biliary cirrhosis was due to a tumor, H.M. having a carcinoma of the pancreas, and A.L. carcinoma of the stomach with metastasis to the liver. In the latter patient a subsequent measurement made thirteen days prior to death, when there was presumably greater liver damage, showed a recovery of 56.5 mg. of pregnanediol.

P.S. (Fig. 5) was first studied after operation for a common-duct stone. With the T-tube in place, the recovery was 77.6 mg.; when the T-tube was removed, the recovery was 84.6 mg. In Patient J.O. (Fig. 6), with obstructive jaundice due to hepatic metastases from a bowel carcinoma, there was a decreased excretion of pregnanediol. This woman was 38 years old and had a concomitant increase in the frequency and duration of the menstrual

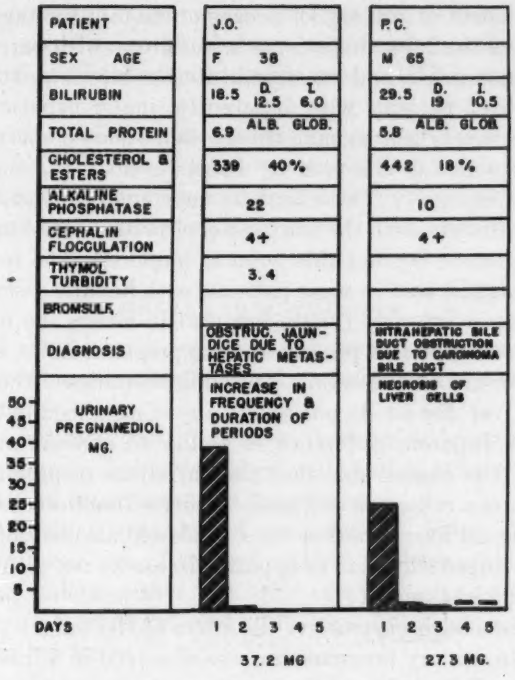

FIG. 6. Decreased excretion of pregnanediol after oral administration of 500 mg. of progesterone, in 2 patients with obstructive biliary-tract disease.

flow. Patient P.C., who also showed a decreased excretion had intrahepatic bile-duct obstruction due to carcinoma of the bile duct with necrosis of liver cells.

DISCUSSION

From the observations in control subjects and the reports of others (10) it can be stated that about 10 per cent of an administered dose of progesterone is recovered as urinary pregnanediol, whether the progesterone be injected intramuscularly, absorbed through the buccal mucosa or given orally. In our control subjects, given 500 mg. of progesterone orally, the

excretion of pregnanediol during the first twenty-four hours did not usually exceed 30 mg. The decreased amounts of pregnanediol recovered in 6 of the patients with parenchymal liver disease suggest that in these individuals the diseased liver was not able to convert progesterone to pregnanediol in the usual amounts. These observations conform to the observations of Masson and Hoffman (4), that in partially hepatectomized rabbits it was necessary to administer orally only 25 mg. of progesterone in order to achieve the progestational effect of 200 mg. of progesterone in intact animals. It is of interest that 1 of the 2 female patients in our group with parenchymal liver disease had amenorrhea and functional uterine bleeding. It is difficult to ascertain why in 3 patients with apparently similar hepatic disorders the pregnanediol recovery was normal whereas in 3 others it was increased. The latter phenomenon was observed by Paschkis *et al.* (8) and by Havens, Myerson and Carroll (7). These authors concluded that chronic liver disease does not interfere with the conversion of progesterone to pregnanediol but that metabolism beyond this point is impaired. The results reported in this study suggest that in some patients with hepatic disease, pregnanediol degradation is interfered with, but that in others the metabolic difficulty is in the conversion of progesterone to pregnanediol or conjugation of pregnanediol for urinary excretion. The clinical studies of the patients with parenchymal liver disease do not reveal any distinguishing characteristics to explain this apparent difference in ability to convert progesterone to pregnanediol. It is conceivable that the variations of pregnanediol excretion observed are a reflection of a more sensitive function than those represented in the usual liver function tests. It should also be noted that measurements of estrogen excretion in hepatic disease do not result in abnormal findings in every instance (11).

In the patients with obstructive disorders of the biliary tract, a significant increase in urinary pregnanediol was observed in 4 instances. The results in Patient B.N. indicate that in the presence of biliary obstruction without diffuse hepatic disease, large amounts of pregnanediol may be excreted in the urine. It has been shown by Hoffman, Masson and Desbarats (12) that rabbits with ligated bile ducts, when given progesterone, excrete an increased amount of pregnanediol in the urine. Our results suggest that this also occurs in human subjects with biliary obstruction. Previous studies from this laboratory have demonstrated that pregnanediol is excreted in bile (6). The present study can be interpreted to indicate that when the biliary tract is obstructed, there is an altered route of excretion, and a greater amount of pregnanediol is found in the urine. It was further noted that when biliary obstruction was relieved and T-tube drainage established the urinary excretion of pregnanediol was diminished.

In 2 patients with obstructive biliary disease there was a decreased re-

covery of pregnanediol after administration of progesterone. In 1 instance there was evidence of hepatic metastases and in the other, necrosis of the liver cells. This suggests that the decreased excretion, even though biliary obstruction was present, was associated with diffuse parenchymal disease. In Patient A.L., who had obstructive biliary cirrhosis associated with metastatic carcinoma, a high excretion of pregnanediol was found. One month later and thirteen days before death, a lesser amount of pregnanediol was excreted, suggesting more hepatic involvement. It is noteworthy that the one female patient in the obstructive group who was still having menstrual periods, had experienced an increase in frequency and duration of her menses.

In view of recent publications (13, 14) concerning the relationship between the liver and the reproductive system, it is of interest that among the 6 females of reproductive age included in this group, 1 had amenorrhea, 1 had alternating amenorrhea and functional bleeding, and 1 had menometrorrhagia.

SUMMARY AND CONCLUSIONS

After the oral administration of 500 mg. of progesterone to patients with parenchymal liver disease, pregnanediol excretion may be decreased, increased or normal.

These results indicate that in some patients with hepatic disease the metabolism of pregnanediol is interfered with, but in others there is a defect in conversion of progesterone to pregnanediol.

An increased urinary excretion of pregnanediol occurs after oral administration of a large dose of progesterone to patients with extrahepatic obstruction. This indicates that in intrahepatic biliary obstruction, there is an altered route of excretion, and an increased amount of pregnanediol may be found in the urine.

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THE DETERMINATION OF COPPER AND ZINC IN NORMAL AND PATHOLOGIC HUMAN THYROID TISSUE*†

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INTRODUCTION

THE literature has been well documented with quantitative studies on the metallic constituents occurring in trace amounts in plant and animal tissues. Many different techniques including chemical, physical and microbiologic, have been utilized in the analyses of these tissues. In many instances the minimal daily requirements for these elements have been established for certain plants and animals. The effects of subminimal and excessive intake levels of these elements in some plants and animals have also been studied.

Because of the lack of adequate quantitative data on the subject of trace elements in human tissues, a study of as many of these elements as possible was begun in our laboratories. At present, qualitative and quantitative analyses of human tissues, both normal and pathologic, are being made. When different techniques were available for the quantitative determination of a single trace element, aliquots of each sample were analyzed by the various methods and the results compared.

In recent years the possible role of copper in thyroid function has been discussed. Weiss (1) reported that supplemental copper and tyrosine were needed for maximal iodination of tyrosine by homogenates. On the other hand, tissue slices incubated in the presence of supplemental copper showed diminished I^{131} uptake. Anaerobiosis almost completely abolished synthesis by tissues, but the activity of homogenates remained relatively unaffected under anaerobic conditions. From these studies it appeared that copper

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was an indispensable constituent of the homogenate system despite its inhibitory effect on the uptake of I^{131} by tissue slices. Fawcett (2) stated that the synthesis which occurred in copper- and tyrosine-supplemented homogenates was due to the formation of monoiodotyrosine. The iodination of tyrosine could not be measured in homogenates supplemented only by copper. Apparently, there was insufficient preformed tyrosine present in the homogenates to allow iodination to proceed at a detectable rate. Hence supplemental tyrosine was needed to provide an adequate substrate for the production of measurable amounts of monoiodotyrosine. The function of cupric ion was considered to be the oxidation of iodide ion nonenzymatically to elemental iodine. The iodine was then accepted by an iodinating enzyme which functioned in the conversion of tyrosine to monoiodotyrosine. Ferric ion or hydrogen peroxide could replace copper in the production of elemental iodine. Taurog *et al.* (3) reported that neither copper nor tyrosine supplements were necessary for synthesis of organic iodinated compounds by thyroid homogenates or thyroid particulate fractions. Cell-free preparations not supplemented with copper and tyrosine converted appreciable quantities of added inorganic I^{131} to labeled proteins. Labeled monoiodotyrosine was found only upon hydrolysis of this protein.

If copper ions were necessary for the normal conversion of iodide ion to elemental iodine, a certain concentration of copper ion in the thyroid should be necessary for normal activity. A study of the copper and zinc content of normal and pathologic human thyroids has been made. A preliminary study of the concentration of these elements in other normal human tissues has also been made.

METHODS

Tissue samples were collected directly from the Department of Pathology at Memorial Center. Samples from outside sources were collected in polyethylene bags, frozen immediately, and transported to our laboratory in the frozen state. All samples were trimmed with a platinum-iridium knife prior to processing.

In order to keep the hazard of contamination at a minimum, the laboratory was equipped with a double door air-lock type entrance and a positive pressure was maintained in the laboratory by means of a filtered forced-air circulation system. All glassware was cleaned with hot nitric acid and the final six rinses were made with reprocessed water (distilled water passed through an amberlite mono-bed ion-exchange resin column).

All samples were wet weighed and dried to constant weight in a vacuum oven for sixty hours at 95° C. Organic material was destroyed by wet ashing in Vycor or Pyrex Kjeldahl flasks, using nitric acid. Ash residues were converted to the chloride form and ash weights obtained. Control blanks were run periodically to check on the purity of cleaning and digesting reagents.

Aliquots of tissue ash were dissolved in hydrochloric acid for colorimetric analyses. Copper and zinc were determined by dithizone extraction (4). Zinc was also determined by means of neutron activation using the Brookhaven National Laboratory reactor. A 20-milligram portion of ash was irradiated in repurified quartz cuvettes. The activated ash was dissolved in aqua regia and diluted to a fixed volume. Aliquots of the activated

TABLE 1. CONCENTRATION OF ZINC AND COPPER IN NORMAL HUMAN TISSUE—MICROGRAMS PER GRAM OF ASH

Tissue	No. of samples	Zinc		Copper	
		Range	Mean	Range	Mean
Adrenal	2	370– 1,010	690	20–206	—
Bladder	2	950– 1,050	1,000	45– 75	60
Bone	2	60– 355	205	14	—
Brain	3	340– 1,220	800	120–220	190
Esophagus	2	950– 1,285	1,115	118–250	185
Gonads	4	790– 1,340	1,070	70–195	128
Heart	9	650– 3,100	1,635	83–525	252
Large bowel	1	620	—	530	—
Small bowel	10	290– 2,300	1,280	60–510	205
Kidney	15	600– 7,540	3,120	90–390	205
Liver	13	2,400– 5,635	4,165	245–840	530
Lung	11	900– 1,370	1,121	90–430	200
Muscle	11	2,460– 5,680	3,660	40–640	305
Pancreas	4	1,300– 2,400	2,010	83–309	145
Prostate	4	2,160–15,900	—	140–805	—
Spleen	14	660– 1,440	1,155	51–340	162
Stomach	4	1,020– 2,400	1,980	170–350	285
Thyroids	8	700– 1,920	1,080	40–160	115

ash were chromatographed on Dowex 50 cation exchange resin columns (5, 6). The activity of the zinc fraction was measured and the zinc content of the ash calculated. When the size of the tissue sample permitted, aliquots of the ash were analyzed by D. C. arc spectrography for both copper and zinc (7).

RESULTS

All normal tissues were from medical-examiner cases of sudden death. No cases were included in which pathologic findings were present at autopsy.

The copper and zinc content of several normal human tissues are sum-

TABLE 2. CONCENTRATION OF COPPER IN HUMAN TISSUE—MICROGRAMS PER GRAM OF WET TISSUE

Tissue	Literature values		Sloan-Kettering Institute values
Thyroid	(Shapiro, 8)	8	2
Rib bone	(Monier-Williams, 9)	15	4
Liver	(Kehoe, 10)	7	11
Liver	(Monier-Williams, 9)	5	—
Liver	(Cunningham, 11)	25	—

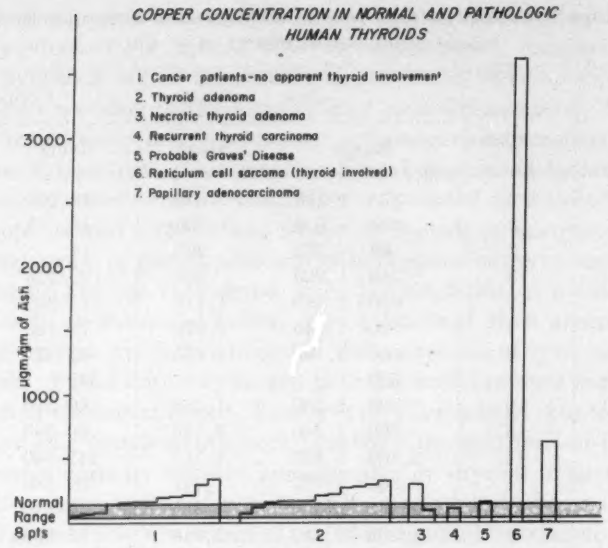

FIG. 1. Group I includes 3 carcinoma of breast, 2 carcinoma of genito-urinary system, 2 carcinoma of extrinsic larynx and 1 of each of the following: adenocarcinoma of rectum, carcinoma of antrum and left eye, carcinoma of pharyngeal wall, carcinoma of nasopharynx and malignant melanoma.

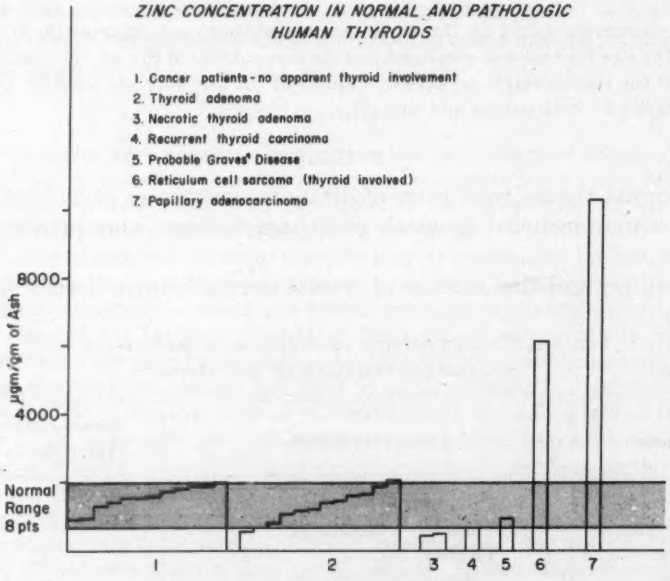

FIG. 2. (For composition of Group 1, *see* legend for Figure 1.)

marized in Table 1. All results were calculated as micrograms/gram of ash. Averages were determined where sufficient data had been obtained.

In Table 2 several values for copper in human tissues as reported in the literature are compared with values obtained in our analyses.

Thirty-six thyroids were analyzed for copper and zinc. Figures 1 and 2 show the results of these determinations. In the Weiss (1) and Fawcett (2) experiments 1 and 5 milligrams respectively of copper per gram of tissue were used. These concentrations are considerably higher than the 2 micrograms per gram of wet thyroid tissue as found in our experiments with human tissue.

DISCUSSION

Considerable controversy exists in the literature regarding the requirement for copper by thyroid homogenates for the production of iodinated tyrosine. The results presented here indicate fairly conclusively that copper cannot function chemically in the oxidation of iodide ion to elemental iodine. On the basis of moles of copper and iodine per gram of wet tissue, the iodine concentration is approximately 1,000 times the copper concentration in the thyroid. The possibility still exists, however, that copper or some other metal may function in the thyroid as an enzyme activator in the organic binding of iodine.

The tendency toward an increased thyroid copper level in patients with cancer in any part of the body cannot be explained at this time. A direct comparison between thyroid activity and thyroid copper levels was not possible in the cases studied, since no good data on the level of activity were available in these cases.

Attempts were made by Nielsen (12) to correlate the serum copper level with the degree of severity of the disease in patients with thyrotoxicosis and myxedema. Patients with basal metabolic rates up to 135 per cent the normal rate (+35 per cent) had normal serum copper levels, whereas patients with metabolic rates greater than 135 per cent normal often had elevated serum copper levels. The 3 cases of myxedema studied by Nielsen had serum copper levels near the lower limit of the normal range. In this same study by Nielsen, attempts were made to correlate variations in metabolic rate with serum copper levels during the course of illness of individual patients; however, no connection could be demonstrated with any degree of regularity.

Cartwright has reported that analyses on various tissues from patients with malignant diseases show marked increases in the copper content of depot organs (13). Increased serum copper levels in acute as well as chronic infections have also been reported by him. However, Cartwright has been unable to confirm the work of Heilmeyer, who reported that agents which

activate the reticulo-endothelial system, such as injections of milk or typhoid vaccine, cause a rise in the serum copper level.

The noted increase in zinc levels in papillary adenocarcinoma and reticulum-cell sarcoma of the thyroid cannot be explained at this time. The slightly decreased values found in the necrotic adenomas are not significantly outside the normal range. Our analyses on other tissues show a higher zinc content in pathologic lung tissue from patients with bronchogenic carcinoma and sarcoma of the breast than in normal lung tissue. On the other hand, in cancer of the kidney the involved areas show a lower zinc content than do uninvolved areas from the same kidney, though both values are within the normal range for that organ.

It would be of interest to determine the thyroid copper level in animals or humans whose thyroid is abnormal and on whom complete studies of thyroid function have been made. In this way a better correlation, should one exist, could be made between copper levels and thyroid activity. However, an increased copper level in hyperactive thyroids may not necessarily prove that copper is essential for iodination of tyrosine in the thyroid. This increased copper level may be only a physiologic response, as is believed to be the case in elevated blood levels in various abnormal states.

SUMMARY

Data have been presented on the copper and zinc content of normal and abnormal human thyroids. A preliminary report on the copper and zinc levels in various other human tissues has also been presented. Adequate interpretation of the results cannot be made at this time, due to lack of complete knowledge of the role of these elements, particularly copper, in human metabolism, both in normal and pathologic states.

Acknowledgments

The spectrographic analyses were performed by N. F. Shimp and Jane Connor, Soils Department, Rutgers University, under a Sloan-Kettering Institute grant.

We wish to thank Dr. Brown M. Dobyns, Department of Surgery, Cleveland City Hospital, Cleveland, Ohio, and Dr. Isabel H. Tipton, Department of Physics, University of Tennessee, Knoxville, Tennessee, for their cooperation in supplying samples for this project.

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THE EFFECT OF IODIDE ON THE RELEASE OF THYROID HORMONE IN HYPERTHYROIDISM*

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INTRODUCTION

THE mechanism whereby the iodide ion beneficially affects the syndrome of hyperthyroidism remains unclear. Wolff and Chaikoff have shown that in the rat, elevation of the serum inorganic iodide concentration to approximately 30 micrograms per 100 ml. inhibits the synthesis of thyroid hormone (1). This effect, however, is transient. Stanley has presented evidence that in man, when the serum inorganic iodide concentration exceeds 6 micrograms per 100 ml. there is partial inhibition of organic binding of iodine in the thyroid gland (2). These experiments suggest that iodine exerts its effect by slowing the rate of synthesis of thyroid hormone. A different process by which iodide may affect hyperthyroidism is intimated by the *in vitro* studies of Albert and associates (3). Their experiments suggest that elemental iodine may reversibly inactivate anterior pituitary thyrotropin (TSH), for when the two are given together, the expected increase in thyroid weight, decrease in thyroid iodine content, and increase in thyroid acinar cell height do not occur. Means and Lerman pointed out similarities between the changes in basal metabolic rate (BMR) of thyrotoxic patients receiving iodine and of myxedematous patients whose maintenance thyroid medication had been withdrawn, and suggested that iodine may exert its therapeutic effect by interfering with the release of hormone from the thyroid gland (4). This hypothesis is supported by DeRobertis, who has offered cytochemical evidence suggesting that iodine interferes with a TSH-controlled proteolytic breakdown of thyroglobulin to active form (5).

Studies have been performed in the hope of clarifying the mechanism of the iodine effect. Observations have been obtained on patients whose thyroids were temporarily incapable of synthesis of hormone, so that any effect of iodine mediated through hormone synthesis could be ignored. The data

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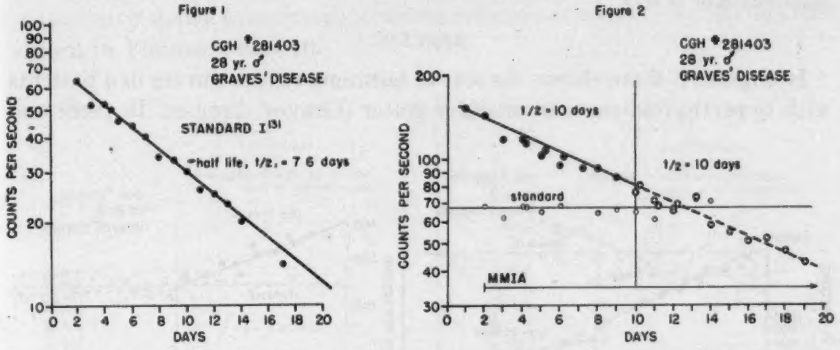
to be reported suggest that, in hyperthyroid man, iodide ion slows the rate of secretion of thyroid hormone, and that this slowing results from interference with release of hormone from the gland rather than with hormone synthesis.

METHOD

Eleven patients with hyperthyroidism were studied. There was no selection as to age, severity of disease, or complications. Seven patients had Graves' disease, with a smooth anodular goiter, and 4 had multinodular goiter with hyperthyroidism. All subjects demonstrated the typical clinical signs and symptoms of the disorder, and all had elevation of the BMR and of thyroidal I^{131} tracer uptake. A tracer dose of approximately



FIGS. 1 and 2. *Control Observations With and Without Methyl Mercaptoimidazole.* Figure 1 shows the results of standard counting; and Figure 2, of *in vivo* counting while the patient was receiving MMIA for a period equal to the duration of subsequent studies. In Figure 2 the curve established during the first ten days of study is continued unchanged during the last ten days. The t/2 in Figure 1 is the observed half-life, whereas that in Figure 2 and subsequent figures is the biologic half-life. Closed circles = observations during control period. Open circles = observations during treatment period. MMIA = 1-methyl, 2-mercaptoimidazole, 40 mg. every 8 hours.

250 microcuries of radioiodine was given orally at the start of each study. Seventy-two hours later, synthesis of thyroid hormone was "blocked" by methyl-mercaptoimidazole (MMIA) administered by mouth continuously in a dosage of 40 mg. every eight hours for a period of sixteen to twenty days. This dosage was chosen because earlier work had demonstrated that administration of MMIA up to 20 mg. every eight hours produced increasing but incomplete "block" of thyroid hormone synthesis as measured by the 24-hour I^{131} uptake (6). However, when MMIA was given in a dosage of 40 mg. every eight hours, and the tracer was administered two hours after the morning dose of MMIA, the 24-hour I^{131} uptake was found to be zero.

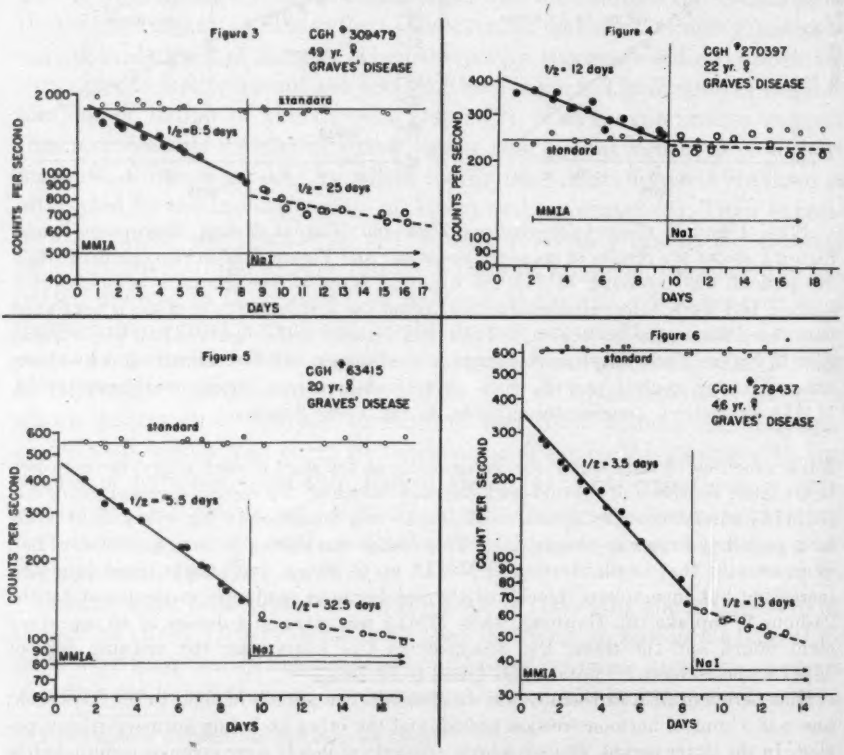
The period of MMIA therapy was divided into two periods of eight to ten days each; one was a control hormone-release period, and the other an iodide hormone-release period. In the latter period, pharmacologic amounts of iodide were given as sodium iodide in a dosage of 50 mg. every twelve hours. During both periods, gamma counts were made twice daily over the thyroid area. In 2 instances anterior pituitary thyrotropin (TSH,

Armour list #3600) was administered simultaneously with the sodium iodide in doses of 15 mg. every twelve hours intramuscularly. The plot of daily counts versus time was depicted on semi-logarithm paper and the slopes of the curves analyzed for statistical differences.

Since the conclusions derived from this study are based on a comparison of slope differences, the consistencies of the counting method and of the control hormone-release curve were first established. Figure 1 shows the observed half-life of a standard dose of I^{131} such as was used for a control of the counting method throughout each study. The curve is highly significant and the observed half-life agrees with the theoretical eight-day half-life within 5 per cent error. In Figure 2 are data obtained from a patient who was given only MMIA throughout both periods. A single straight line was obtained. Therefore, there was but one rate of hormone release constantly observed during the administration of MMIA.

RESULTS

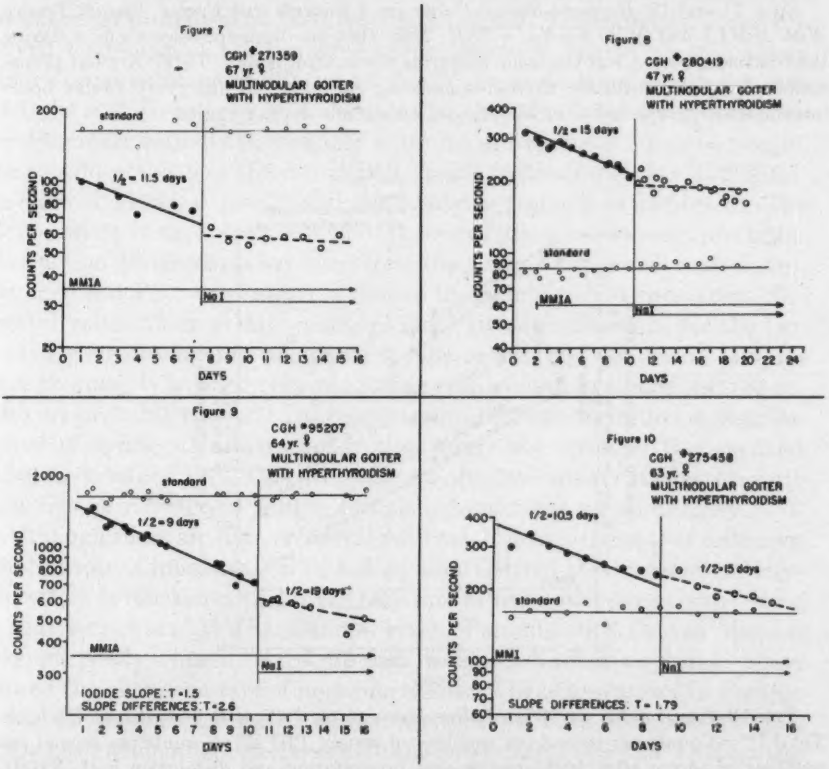
In Figures 3–6 are shown the sets of hormone-release curves in 4 patients with hyperthyroidism and anodular goiter (Graves' disease). In these and



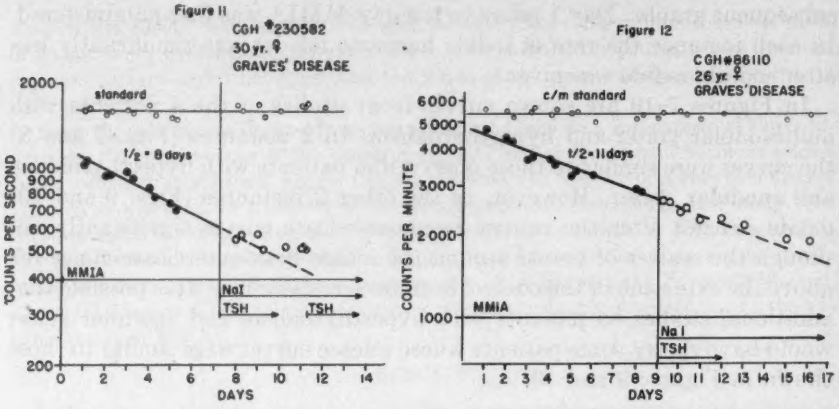
FIGS. 3, 4, 5 and 6. *Hormone-Release Curves for 4 Patients with Graves' Disease Treated with MMIA and MMIA+NaI.* NaI = Sodium iodide in a dosage of 59 mg. every twelve hours. For key to other symbols, *see* Figures 1 and 2. *See* text for further explanation.

subsequent graphs, Day 1 refers to the day MMIA was first administered. In each instance the rate of iodide hormone release was significantly less after sodium iodide was given.

In Figures 7–10 are shown curves from studies of the 4 patients with multinodular goiter and hyperthyroidism. In 2 instances (Figs. 7 and 8) the curves were similar to those observed in patients with hyperthyroidism and anodular goiter. However, in the other 2 instances (Figs. 9 and 10) iodide did not alter the control hormone-release curves significantly, although the scatter of points around the iodide hormone-release curve fell above the extension of the control hormone-release curve. It is possible that additional studies on patients with hyperthyroidism and anodular goiter would have shown some patients whose release curves were similar to those shown in Figures 9 and 10.



FIGS. 7, 8, 9 and 10. *Hormone-Release Curves for 4 Patients With Multinodular Goiter and Hyperthyroidism.* The data are presented as in Figures 3 through 6. Note that in Figures 9 and 10 the difference between the MMIA and the MMIA+NaI slopes is not significant. For key to symbols, *see* legends of prior figures.



FIGS. 11 and 12. *Hormone-Release Curves for 2 Patients with Graves' Disease Treated With MMIA and MMIA+NaI+TSH.* Note that the hormone-release curve during the treatment period was the same as during the control period. TSH = Armour preparation of anterior pituitary thyroid-stimulating hormone, 15 mg. every twelve hours intramuscularly. For key to other symbols, *see* legends to prior figures.

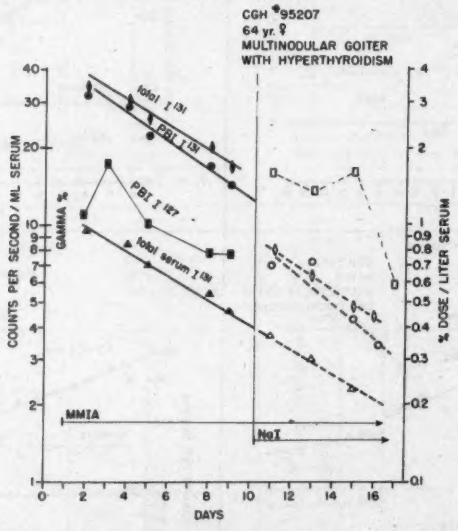


FIG. 13. *Observations on Serum Iodine Levels in the Patient Represented in Figure 9.* Total I^{131} = Counts per second per milliliter of serum. PBI I^{131} = Counts per second per milliliter of serum after trichloracetic-acid precipitation and dissolution with NaOH. PBI I^{127} = Gamma per 100 milliliters of chemically determined serum protein-bound iodine, using a modification of Barker's method. Total serum I^{131} = per cent of dose per liter of serum. For key to other symbols, *see* legends to Figures 1–6.

In Figures 11 and 12 are shown curves wherein, during the iodide hormone-release period, TSH was given concomitantly with iodide. The control and iodide-plus-TSH curves were not significantly different: as soon as TSH was discontinued the curves became more horizontal.

DISCUSSION

The data presented here support the hypothesis that iodide slows the rate of release of hormone from the thyroid in patients with hyperthyroidism. The argument of Ansell and Miller that the results of a study such as that represented in Figure 3 could be explained by assuming a small "leak" in the MMIA "block" still poses a problem (7). During the studies depicted in Figures 3 and 9 several blood samples were drawn during the control and iodide periods. The sera obtained were analyzed for total I^{131} content, for I^{131} content of the trichloracetic-acid precipitable fraction, and for chemical protein-bound iodine (PBI) content. Theoretically, if the observed flattening of the hormone-release curve is due to an incomplete "block" and subsequent dilution of the I^{131} hormone-label within the thyroid, the specific activity of the serum PBI should fall. However, if the observations are actually the result of a slowing of the rate of hormone release, the specific activity of the serum PBI should remain constant. Both studies showed identical results and one study is graphed in Figure 13. The radioactivity in the total serum and the serum trichloracetic-acid precipitable fraction decreases, as expected from the thyroid decay curve. However, the chemical PBI concentration during the iodide period rises above the control values in an erratic manner. The PBI determinations for the two studies were obtained in independent laboratories and the serum samples were thoroughly washed to avoid iodide contamination; therefore, the results are probably correct.[1] The rise in serum PBI concentration during the period of iodide administration is most likely the result of the so-called "Danowski effect" (8). This precludes an absolute answer to the theoretic argument of Ansell and Miller, but their hypothesis seems unlikely.

Although there are data on only 2 patients, it is of interest that following simultaneous administration of iodide and TSH to the hyperactive thyroid which is blocked with MMIA, the rate of hormone release is the same as that seen when MMIA alone is given. Patients with Graves' disease were purposely chosen, since all such patients studied on iodide alone showed the change in rate of hormone release. The observations in Figures 11 and 12 may be explained in either of two ways. First, it is possible that iodide in some manner interferes with the accelerating effect of TSH on the release of thyroid hormone (6). As the TSH was exogenous, it may be as-

[1] The authors wish to thank Dr. John Stanbury and Dr. Belton Burrows for performing these determinations.

sumed that the interference occurred outside of the hypothalamus-anterior pituitary pathway; consequently it might occur either along the anterior pituitary-thyroid pathway or at the level of the thyroid cell. If the interference were at the cell level, iodide could impede only the release of hormone, since MMIA had already inhibited its synthesis. Second, it is possible that TSH in some manner interferes with the effect of exogenous iodide on thyroid hormone release. Since the amount of iodide given in the present studies exceeds the amount ordinarily necessary to induce a satisfactory iodide response in hyperthyroidism with anodular goiter, it seems illogical to presume that TSH interferes with the circulating iodide ion before the iodide reaches the thyroid (9). Accordingly, the thyroid acinar cell seems the most likely site of iodide effect, and the TSH-thyroid gland pathway seems the most likely mechanism interfered with, in order to produce this effect. Shellabarger and Godwin concluded that thyroxine and triiodothyronine affected the usual response of the thyroid to TSH in the same manner (10).

There may be a correlation between the results reported here and the established clinical impression that patients with Graves' disease respond more rapidly to all forms of therapy than do patients with multinodular goiter and hyperthyroidism. It is possible that TSH plays a more fundamental role in the pathogenesis of Graves' disease than in the pathogenesis of multinodular goiter with hyperthyroidism. This explanation suggests two diseases: 1) a hyper-anterior-pituitarism, with rapid appearance of thyroid changes and the clinical syndrome described by Graves, and 2) a hyper-thyroidism, with gradual appearance of symptoms. It is well known that the latter syndrome differs from that described by Graves, in that it is manifested chiefly by cardiovascular signs and symptoms.

There is some experimental basis other than that herein reported for thinking of thyroid disease in this way. Greer postulated two distinct thyrotropins to explain the lack of a direct relationship between thyroid growth and thyroid iodide-trapping (11). Halmi and associates investigated this idea further and concluded that "other factors" in addition to TSH affect the thyroid iodide-trapping mechanism (12). VanderLaan and Caplan, following this line of investigation, concluded that one of these "other factors" affecting the thyroid iodide-trapping mechanism in hypophysectomized rats receiving constant exogenous TSH is the total thyroidal iodine concentration (13). Werner has argued frequently that the disease hyperthyroidism originates within the thyroid gland (14). On the other hand, there are so many data linking the anterior pituitary to the thyroid as a stimulating force that it is difficult to accept Werner's explanation as the sole, or even the most common pathogenesis of hyperthyroidism. An hypothesis assuming at least two pathogenetically different types of hyperthyroidism seems warranted.

CONCLUSIONS

1. Iodide slows the rate of release of formed hormone from the toxic human thyroid.

2. This effect of iodide probably is the result of some interference with TSH activity at the level of the thyroid gland.

Addendum

Studies by Drs. David H. Solomon and Monte Greer on euthyroid patients, using a method rather similar to that employed in our study, were reported at the 1954 meetings of The Endocrine Society and of The Thyroid Study Group, respectively. These workers presented data very similar to those herein reported.

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A SIMPLIFIED DISTILLING HEAD FOR THE MEASUREMENT OF CORTICOSTEROIDS BY THE FORMALDEHYDOGENIC METHOD

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THE measurement of urinary corticosteroids by the formaldehydogenic method of Daughaday, Jaffe and Williams (1) has gained wide acceptance in the routine assessment of adrenocortical function. The distillation of the formaldehyde formed by the periodic-acid oxidation of the steroids bearing a C-21 primary alcohol group is a time-consuming step and, in addition, requires expensive equipment. According to this technique, the solution containing the formaldehyde is transferred to a three-piece distilling apparatus and, after the distillation is completed, the three pieces must be disassembled, thoroughly cleaned, reassembled and the next sample introduced. If two units are run simultaneously, one technician can distill 6 samples in one hour. In our laboratory, approximately 84 per cent of the formaldehyde can be recovered.

A piece of apparatus similar to that described by Stotz (2) for the distillation of acetaldehyde was constructed,[1] except that the condenser was eliminated and a pipette safety-trap was introduced to prevent the distilled solution from sucking back into the flask (Fig. 1). The oxidation and distillation are accomplished in a 50-ml. round-bottom flask with a 19/38 standard taper joint. The suggestions of Daughaday *et al.* (1) with regard to the quantities of solutions and reagents are followed.

The head is attached by its tapered joint to the flask, a drop of 15 M sulfuric acid is used as a sealer, and a 15-ml. Pyrex graduated centrifuge tube containing 1 ml. of 4 per cent sodium sulfite solution is the receiver. The centrifuge tube with the delivery tube of the distilling head dipping

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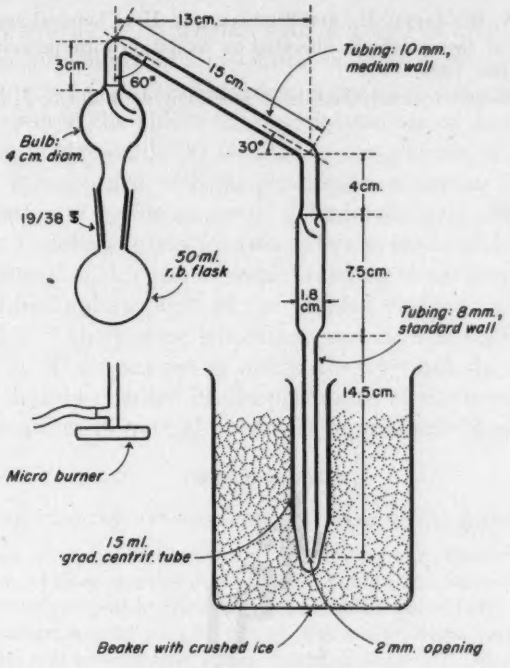
[1] Manufactured by the Lanning Glass Company, Vineland, New Jersey. (Cost per unit, $5.00.)

below the surface of the sodium sulfite solution is surrounded by a beaker containing crushed ice in water. The flask is heated with a microburner. Once heating has started, it should not be interrupted, although the pipette arrangement will prevent any sucking back and consequent loss of the sample. This has never occurred in the course of distillation of over 400 specimens. When a 5-ml. sample has been distilled, the head is removed,



APPARATUS FOR DISTILLING FORMALDEHYDE

FIGURE 1

rinsed thoroughly with hot tap water and then with distilled water. The distilling flask must be rinsed immediately in order to prevent scarring by the concentrated sulfuric acid left behind.

In practice, when six distilling heads are being employed, two distillations proceed simultaneously, two heads are being washed and two heads are draining. Since each distillation requires less than three minutes, one technician can distill at least 20 samples in one hour. Approximately 98 per cent of the formaldehyde can be recovered with this distilling head.

SUMMARY AND CONCLUSIONS

A simple and inexpensive piece of apparatus for the distillation of formaldehyde generated by periodic-acid oxidation of urinary corticosteroids has been described, which allows the recovery of approximately 98 per cent of this material within the course of three minutes.

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GOITER FROM PROLONGED INGESTION OF IODIDE*

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THE development of myxedema and/or goiter in children and adults during the prolonged administration of large doses of iodide has recently been reported (1, 2, 3). This phenomenon has been attributed to an antithyroid action of the iodide (2). Administration of desiccated thyroid (2) or cessation of the iodide (3) resulted in a regression of the symptoms.

This report is concerned with the production of similar symptoms from the administration of iodide as syrup of hydriodic acid (SHI), potassium iodide (KI) or Lugol's solution for two to five years to asthmatic patients. The patients were 7 males and 6 females ranging in age from 5 to 17 years (Table 1). All had enlargement of the thyroid varying from Grade II to Grade V, and in 7 there were laboratory and clinical findings consistent with myxedema. The cessation of iodide therapy and the administration of desiccated thyroid resulted in disappearance of the myxedema and regression of the goiter to normal or almost normal size in each patient.

CASE REPORTS

The following case reports are representative of the group:

Case 1. D. J., a white boy aged 7½ years, was first seen in March 1954 because of a tumor in the neck, of three months' duration. The patient had had asthma all of his life and had been taking syrup of hydriodic acid (¾ teaspoon twice daily) for the preceding five years. His weight was 52 pounds; height, 48½ inches; blood pressure, 90/70; and pulse, 100. The thyroid was diffusely enlarged (about Grade III), smooth and firm. The circumference of the neck was 26 cm.

The laboratory reported a normal hemogram, a serum cholesterol level of 187 mg. per 100 ml., and a serum protein-bound iodine (PBI) level of 16.1 mcg. per 100 ml. The thyroidal uptake of I^{131} was 3.4 per cent, and urinary excretion was 90.1 per cent of the administered dose at twenty-four hours. Roentgenograms of the chest revealed substernal extension of the thyroid gland, and those of the extremities showed normal skeletal development.

Iodide was discontinued and desiccated thyroid, ½ grain daily, was prescribed. At the

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end of one month the dose was increased to 1 grain daily. At six months the child had gained 2 pounds and had grown 2½ inches; the blood pressure was 100/70 and the pulse was 110. The tachycardia could be explained by the fact that the patient was taking ephedrine for a mild attack of asthma. The gland had regressed to Grade I status. The concentration of serum cholesterol was 141 mg. per 100 ml. and of PBI, 8.6 mcg. per 100 ml.; thyroidal uptake of I^{131} was 11.7 per cent, and urinary excretion was 74.5 per cent in twenty-four hours. Chest roentgenograms were normal. After one year of thyroid therapy, his weight was 58 pounds; height, 51¾ inches; blood pressure, 80/50; and pulse, 80. The neck circumference had decreased to 20 cm. The thyroid was less than Grade I in size. The concentration of serum PBI was 6.6 mcg. per 100 ml., and of cholesterol, 166 mg. per 100 ml. Tracer studies with I^{131} revealed an uptake of 12.6 per cent and a urinary excretion of 69 per cent. The desiccated thyroid was discontinued.[1]

Case 2. K. M., a white girl aged 10 years, was referred in June 1954 because of goiter. She had been taking syrup of hydriodic acid (1 teaspoonful three times daily) for asthma during the preceding five years. The goiter was first noticed two years previously or three years after beginning iodide therapy. Desiccated thyroid (1 grain daily) and Lugol's solution, (10 drops daily) were administered for one year without improvement. Thyroid had been discontinued three months before referral, but the Lugol's solution had been continued. Her weight was 79 pounds; height, 56½ inches; blood pressure, 90/70; and pulse, 100. The thyroid was horseshoe-shaped, diffusely enlarged, Grade III, smooth, and moderately firm. Iodide-induced goiter was suspected, and the patient was advised to stop all medication for thirty days and then to return for laboratory studies.

After this thirty-day deprivation of iodide, the thyroid had regressed only slightly in size. The hemogram and urinalysis findings were within normal limits; the basal metabolic rate, plus 13 per cent; serum cholesterol level, 260 mg. per 100 ml.; serum PBI level, 14.4 mcg. per 100 ml.; thyroidal uptake of I^{131}, 14.3 per cent; and urinary 24-hour excretion, 64 per cent of the dose. Roentgenograms of the extremities showed normal skeletal development. Desiccated thyroid, 1 grain daily, was prescribed. Three months later the patient's weight was 78½ pounds and her height was 58½ inches—a gain of 2 inches. The blood pressure was 120/80 and pulse, 100. The thyroid was barely palpable. The laboratory at this time reported the concentration of serum cholesterol to be 177 mg. per 100 ml., and PBI, 5.7 mcg. per 100 ml.; tracer studies with I^{131} revealed a thyroidal uptake of 11.6 per cent and urinary excretion of 68.1 per cent in twenty-four hours. Desiccated thyroid, 1 grain daily, was continued. She was next seen two months later, in February 1955, at which time the thyroid medication was discontinued.

In April 1955, her weight was 88 pounds; height, 59 inches; blood pressure, 100/70; and pulse, 80. Thyroid had been omitted for five weeks, and iodides for eleven months. The thyroid was considered normal in size and the patient was feeling unusually well. The uptake of I^{131} was 27.7 per cent and urinary excretion was 61.3 per cent in twenty-four hours.

Case 3. C. McF., a white girl aged 8 years, was referred on June 17, 1950 because of goiter. She had been taking 3 teaspoonfuls of syrup of hydriodic acid daily for the preceding four years because of asthma. About one year previously, the goiter had been observed and ½ grain of desiccated thyroid daily prescribed and the iodide continued. The thyroid increased in size and at the time of our examination was diffusely enlarged to approximately Grade 3.5. The gland was moderately firm. An apparent adenoma,

[1] Six months later the patient was clinically well, the thyroid was normal to palpation, and laboratory studies showed normal thyroid function.

about 1.5 cm. in size, was palpated at the right superior pole. The child presented clinical signs of myxedema.

Because of the apparent adenoma, subtotal thyroidectomy was performed without interruption of treatment, and a large (80 Gm.) multicystic gland was removed. No adenoma was found. The histologic section showed marked hyperplasia with lack of colloid formation.

Following thyroidectomy and the administration of 1 grain of desiccated thyroid daily, the patient's progress has been normal for the past four years.

RESULTS

I^{131} uptake by the thyroid: The activity of the thyroid in 5 of the patients was evaluated by the ability of the gland to accumulate I^{131} (Table 1). The tests were carried out on Patients D. J. and B. C. before iodide was stopped. Their thyroids showed a low uptake of I^{131}, typical of that seen during iodide therapy. On the other hand, the thyroidal uptake in the patients in whom iodides had been omitted approached that of normal. Patient K. M., four weeks after cessation of iodide therapy and before desiccated thyroid therapy had been initiated, showed a nearly normal I^{131} uptake, but the serum PBI level was still high. Furthermore, following the administration of 60 mg. of desiccated thyroid per day for ninety days there was the expected slight drop in the I^{131} uptake, and five weeks after discontinuation of the desiccated thyroid both the uptake and the serum PBI level were in the euthyroid range. Tracer studies on Patient B. B. yielded normal results two months after cessation of the iodide therapy.

Patient G. T. was of particular interest because, unlike patients in some previous reports (3), his thyroid continued to enlarge for two years after cessation of iodide therapy. Furthermore, following administration of a tracer dose of 15 μc. of I^{131} the thyroidal uptake was 41.2 per cent in twenty-four hours; repetition of the test ten days later showed essentially the same result—44.2 per cent in twenty-four hours. The values were in the range of borderline hyperthyroidism and indicated an increased demand for iodine by the gland. The normal serum PBI level (5.3 mcg. per 100 ml.), the lack of clinical signs of thyrotoxicosis, and the persistence of the goiter suggested that the thyroid had partly but not completely overcome the blocking effect of the previously administered iodide on the synthesis of thyroxine. It is assumed that over-production of thyrotropin had continued, in spite of the withdrawal of the iodide for two years.

Histology of the goiter: Thyroidectomy was performed on 1 patient, C. McF. (Table 1; Case Report No. 3) because the large goiter seemed to contain an adenoma. Histologic examination showed hyperplasia with little or no colloid formation. The picture was similar to that seen in glands of patients receiving thiouracil and in those of goitrous cretins.

Thyroid hormone administration: In every patient (Table 1) the administration of thyroid either as desiccated thyroid (T), 15–60 mg. per day, or as sodium *l*-thyroxine (SLT), 0.1–0.4 mg. per day, was associated with a

TABLE 1. EFFECT OF DESICCATED THYROID ON IODINE-INDUCED GOITER

Patient			Iodide Therapy			Thyroid Activity				Thyroid Therapy*		
Initials	Age (yrs.)	Sex	Dose of iodide per day	Duration (yrs.)	Size of Goiter	I^{131} Uptake (% dose /24 hrs.)	I^{131} Excretion (% dose /24 hrs.)	Serum PBI (mcg. /100 ml.)	BMR %	Dose (mg./day)	Duration (wks.)	Size of Goiter
			Syrup HI (ml.)									
DJ	7.5	M	8	5	4+	3.4	90.1	16.1	−18	T-60 SLT-0.1	24 20	1+ <1+
KM	10	F	15	5	3+	14.3†	64.0†	14.4†	−12	T-60	28	<1+
BC**	5.5‡	M	10	2.5	3+	4.8	95.0	11.1	+ 6	T-30	32	<1+
GT	17	M	15	3	3+	41.2§ 44.2	52.0§ 49.4	5.3	− 3	SLT-0.4	12	<2
CMc**	8	F	15	3.5	4+	—	—	—	−12	OP	—	0
JS	5	F	10	2.5	2+	—	—	—	—	T-15	52	<1+
BM	7	F	10	2	3+	—	—	—	−10	T-15	61	0
SB	10	F	15	2	3+	—	—	—	—	T-30	32	0
RK**	15	M	15	2.5	2+	—	—	—	−32	T-60	28	0
			KI (mg.)									
EK**	9	M	236	2	2+	—	—	—	—	T-30	28	0
PM**	16	F	900	4	3+	—	—	—	—	T-60	62	1+
BE**	17	M	1500	5	5+	—	—	—	−30	T-60	8	>1+
			Lugol's (min.)									
BB**	11	M	30	3	2+	26‖	55	—	− 3	T-130 SLT-0.2	40 20	<1+

* T = desiccated thyroid; SLT = sodium *l*-thyroxine; OP = thyroidectomy.
** Clinical myxedema.
† One month after stopping syrup of hydriodic acid.
‡ Skeletal age, 3 years.
§ Two years after stopping iodide.
‖ Two months after stopping iodide.

regression in the size of the goiter which was evident within eight weeks of treatment.

DISCUSSION

The development of myxedema and/or goiter in these patients after prolonged administration of large doses of iodide, and the remission of these signs and symptoms after stopping iodide therapy and instituting thyroid therapy is in agreement with the findings in previous reports (1, 2, 3). It seems that an excess of iodide, as well as a lack of iodide, will result in a diminished production of hormone by the thyroid. The excess supply of iodide apparently interferes with the mechanism for the synthesis of the thyroid hormone. Fawcett and Kirkwood (4) made the interesting suggestion that excess iodide traps the elemental iodine, possibly as I_3, thus making it unavailable for iodinating tyrosine.

Although the incidence of myxedema and/or goiter is small among the great numbers of asthmatics and others who take iodides without interruption for prolonged periods, it is greater than in the general population. It is presumably a physio-pharmacologic effect, probably the result of a unique or peculiar response in the thyroids of these patients.

SUMMARY

Goiter and varying degrees of hypothyroidism developed in 13 patients who had taken iodide for two to five years for control of asthma. In each instance, upon discontinuation of iodide and administration of desiccated thyroid or sodium *l*-thyroxine, the thyroid regressed in size to normal or near normal, and the patient became euthyroid. From the evidence available at present, it is concluded that in some individuals prolonged iodide therapy may have an antithyroid action similar to that of thiouracil.

Addendum

Since this paper was presented we have observed 3 additional patients with similar histories and findings, in whom the goiter regressed to normal or near normal following discontinuation of iodide and without administration of thyroid hormone.

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Teaching Clinic

SPORADIC GOITER OF THE NEWBORN*

WHERE goiter is endemic, the frequent occurrence of goiter in the newborn infant has been well documented. Sporadic goiter of the newborn has been less frequently noted. That it is not a rare condition is suggested by the fact that during the past two years 3 newborn infants with goiters have been admitted to the Children's Hospital of Philadelphia.

CASE REPORTS

Case 1. **KN,** a white female infant, was admitted at the age of 19 days because of a swelling in the neck since birth, choking spells associated with feeding for five days, and weight loss of 18 ounces since birth. This baby was the product of the third pregnancy of a 38-year-old nurse, who had been treated with stilbestrol for the first seven months. During the fifth month of pregnancy she had been hospitalized because of nervousness and tachycardia. Her BMR was over +50 per cent. Lugol's solution (10 drops daily), propylthiouracil (300 mg. daily), and digitalis were administered. Lugol's solution was continued throughout pregnancy but the propylthiouracil was discontinued after two months and the digitalis after one month. The full-term delivery was uncomplicated.

Physical examination on admission revealed a well developed, irritable infant in mild distress. The pulse rate was 250 per minute and the blood pressure 80/40. There was a collar-like mass in the neck, extending laterally to the angles of the mandible. The neck veins were distended. The liver was palpable 1½ cm. below the costal margin. Results of laboratory studies were within normal limits, except for a serum cholesterol level of 85 mg. per 100 ml. and a protein-bound iodine level (PBI) of 18.7 micrograms per 100 ml. The bone age was commensurate with that of a normal newborn white infant. By x-ray examination, a large soft-tissue mass in the retropharyngeal space was seen to displace the trachea anteriorly. The infant was treated with digitalis and oxygen for the first forty-eight hours. The pulse rate decreased to 160–180, but diarrhea with occasional vomiting occurred. Propylthiouracil (5 mg. every six hours) and Lugol's solution (5 drops every six hours) were begun. The infant gradually improved and gained weight. The goiter diminished in size. Lugol's solution was discontinued after two days and propylthiouracil after one week. The findings on physical examinations at the ages of 2 months and 2½ years were normal.

Case 2. **BR,** a white female infant, was admitted on the day of birth because of a swelling in the neck, dyspnea and cyanosis. Since the second month of the pregnancy, the 26-year-old mother had been treated for possible hyperthyroidism (BMR, +32 per

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cent) with 5 grains of potassium iodide three times daily. Delivery at term was uncomplicated. At birth, a large mass in the neck of the infant was noted.

Physical examination on admission disclosed a large, smooth, symmetrical goiter extending laterally beyond the sternocleidomastoids. There was moderate dyspnea and slight cyanosis. The blood pressure was 90/60, and the pulse rate 140. Laboratory studies showed a serum cholesterol level of 144 mg. per 100 ml. The admission serum PBI level was "too high to read"; on the fourteenth day it was 17.5 micrograms per 100 ml., and on the twenty-eighth day 14.8 micrograms per 100 ml. Bone age was normal. Roentgenograms of the neck demonstrated a soft-tissue mass surrounding and compressing the trachea; widening of the superior anterior mediastinum was noted and thought to be due to a substernal extension of the goiter. The infant was placed in oxygen. Without other treatment, the goiter gradually diminished in size and within two weeks respiration and swallowing were normal. No physical abnormalities were noted on re-examination at the age of 2 years.

Case 3. **MN,** a white female infant, was admitted at the age of 12 hours because of a mass in the neck and cyanosis. The 27-year-old mother had had 5 previous pregnancies. Throughout this uneventful pregnancy, the mother had taken calcium, viosterol and occasionally barbiturates and antacids, but no known goitrogenic foods or drugs. There had been no symptoms or signs suggestive of maternal thyroid disease.

On physical examination, the infant was dyspneic and cyanotic. There was suprasternal, intercostal and substernal retraction. A large collar-like symmetrical mass with a palpable isthmus was noted in the neck. There were moist rales in the lungs. Immediate bronchoscopic examination revealed marked compression of the trachea anteriorly and laterally at the level of the thyroid. A thyroidectomy was performed on the first day of life and approximately 80 per cent of the thyroid (13 grams) was removed. Postoperatively the infant had mild stridor. On the third day of life, the serum PBI level was 6.3 micrograms per 100 ml. On the tenth day of life the level of serum calcium was 6.3 mg.; of phosphorus, 8.2 mg.; and of cholesterol, 153 mg. per 100 ml. Two weeks post partum the maternal serum PBI concentration was 10.5 micrograms per 100 ml. Microscopic examination of the thyroid tissue showed "large, very irregular, convoluted acini, some of which contained a small amount of colloid. The lining cells varied greatly in size and shape. The nuclei were pleomorphic, hyperchromatic and at times of bizarre appearance." The diagnosis: "Undiagnosed lesion of the thyroid." On discharge the baby was receiving calcium chloride and Drisdol, which were discontinued at the age of 2 months. At 1 month of age, marked circulatory mottling, slight puffiness of the face, enlargement of the tongue and constipation were noted. The level of serum calcium was 9.0 per 100 ml.; of phosphorus, 6.7 mg. per 100 ml. and of PBI, 1.9 micrograms per 100 ml. A diagnosis of postoperative hypothyroidism was made and desiccated thyroid was administered.

DISCUSSION

A partial review of the pertinent literature is summarized in Table 1.

Etiology. Goiters have been observed in infants born of normal mothers, of those with suspected hyperthyroidism or hypothyroidism, of those who received thiouracil derivatives or iodides, and of those who did not receive any known goitrogens. Many case reports are poorly documented, often probably due to the emergency nature of the infant's status and the need for immediate treatment. In many instances no mention is made of the

TABLE 1. SPORADIC GOITER OF THE NEWBORN*

Author	Mother		Infant					
	Thyroid status	Rx	Thyroid status	Dyspnea; cyanosis	Misc. signs	Surgical Rx	Thyroid histology	Course
Peterson (1)	U	U	U	+		Thx	Adenoma; colloid degen.	
White (2)	Hy	U	Hy?		E+T			Died 25 hrs.
	Hy	U	U		E		Hyperplasia	Stillborn
McLanahan (3)	Simple goiter	U	U					Stillborn
Mitchell (4)	U	U	U	+		Trmy (2 mos.)	Hyperplasia	Died 3 mos.
Williamson (5)	N		Hy?		E+T	Thx (18 mos.)		Continuous enlargement 18 mos.
Solis-Cohen (6)	U	O	U	+				Died 48 hrs.
Parmelee (7)	Asthma & goiter	I	N	+				Normal 1 yr.
	Hy	I	N?	+				Normal 1 yr.
	N	I	N	+				Normal 1 yr.
Davies (8)	Asthma	O	?	+			Hyperplasia	Died 14 d.
	N	O	?	+			U	Died 27 d.
	3 pregnancies	O	?			Thx	Hyperplasia	Well at 9 mos.
Eaton (9)	Hy	Th	N?					Normal 3 mos.
Davis (10)	Hy	Th	U		E			Stillborn
Kunstadter (11)	N?	U	Ho?	+		Trmy	Hyperplasia	Died 32 hrs.
Verel (12)	Hy	Th	N					Normal
Seligman (13)	Hy	Th	?	+		Trmy		Died
		I						
Altman (14)	N?	O	Hy?			Thx		Hyperthyroid at 2 mos.
Jones (15)	N	O	?	+			Hyperplasia	Died 30 min.
	N	O	?	+			Hyperplasia	Died 7 hrs.
Schifrin (16)	N	O	Hy?	+	E	Thx		Thyroid Rx
Fischer (17)	Hy	Th						
		I	?	+	E			Well at 4 mos.
Keynes (18)	Hy	Th	Ho?	+				Recovered
	Hy	O	Hy?		E			U
Saye (19)	Hy	Th						
	(Twins)	I	U		E			Stillborn
			?		E			Recovered
Elphinstone (20)	Hy	Th	Hy?	+				Recovered
Morris (21)	Hy	Th	Ho?					Hydrocephalus
Salm (22)	Hy	Th	U				Hyperplasia	Stillborn
Koerner (23)	Ho	Thyroid	Hy		E+T			Recovered
Hardyment (24)	N							
	Asthma	I	N		E			Normal 3 mos.

* U=unknown or not stated. Th=thiouracil derivatives. I=iodides. Hy=hyperthyroid. Ho=hypothyroid. N=euthyroid. ?=Undecided. Trmy=tracheotomy. Thx=thyroidectomy. E=exophthalmos. T=tachycardia.

maternal thyroid status; in others, modern diagnostic criteria of maternal thyroid function, such as determination of the serum PBI level, were not employed. These are unfortunate omissions, because many authors nevertheless attribute neonatal goiter to disturbances of maternal thyroid function. One author (23) postulates that maternal hypothyroidism leads to neonatal goiter as a result of transplacental transmission of maternal thyrotropin, and several others believe maternal hyperthyroidism to be the cause. Animal experiments (25), at least, do not support the former assumption, for thyroidectomy of the pregnant rat does not induce hyper-

plasia of the fetal thyroid. Again, it would be difficult to explain goiter in the infant born of a thyrotoxic mother on the same basis, since the measurable level of circulating thyrotropin is apparently low in thyrotoxic states; it is probably not coincidence that in all reported instances except one (2), a goiter was found only after treatment of the thyrotoxic mother with thiouracil drugs or iodides.

The thiouracil derivatives are well known goitrogens. There are probably other goitrogenic drugs and foods not as yet recognized. Propylthiouracil given to the pregnant rat leads to hyperplasia of the fetal thyroid (25). Presumably in such experiments and in the reported human cases the goitrogen reaches the fetal circulation and directly inhibits the synthesis of thyroxine by the fetal thyroid; this in turn leads to increased production of fetal thyrotropin and thyroid hyperplasia. When the gland is removed from the influence of thiouracil a transient, self-limited episode of neonatal hyperthyroidism may occur. Although this postulated sequence of events seems eminently logical, it is not at all clear why so few infants born of mothers treated with thiouracil drugs have recognizable goiters during the neonatal period.

Iodine deficiency in the mother is frequently associated with neonatal goiter in certain areas of the world. Few of the sporadic goiters listed in the table would seem to be related to this cause. Aside from a few published cases of thyroid neoplasia (26), the type of goiter found is a diffuse hyperplasia rather than an accumulation of colloid, as in iodine deficiency (? case of Peterson (1)). Many of the reported cases appear to be related to the opposite condition, namely, excessive intake during pregnancy of iodine prescribed for real or suspected hyperthyroidism or for other diseases, such as asthma. This relationship occurs in species other than man. No ready explanation is available, since iodides are not otherwise known to be goitrogenic. It is possible that the fetal thyroid, with an increased avidity for iodides which is perhaps even greater than that of the newborn thyroid (27), reacts in a different fashion.

Diagnosis. It is difficult to determine from the published reports the true functional state of the goitrous neonatal thyroid. The diagnosis of hyperthyroidism was made in several instances on the basis of clinical signs (tachypnea, tachycardia, exophthalmos and tremor) which may have resulted instead from compression of the trachea and neck vessels by an enlarged thyroid. Diarrhea alone during the neonatal period occurs too frequently from other causes to be used in support of this diagnosis. In only a few cases was the serum cholesterol level determined and in none was the serum PBI level determined prior to treatment. Our first case presented most of the expected physical signs of transient neonatal hyperthyroidism. The very low concentration of serum cholesterol and the high concentra-

tion of serum PBI support this diagnosis, but it is possible that the maternal ingestion of iodides until the time of delivery artificially raised the latter level. The same objection might be made to the diagnosis of hyperthyroidism in the second infant. However, the persistent elevation of the PBI level on the nineteenth day of life in Case 1 and on the twenty-eighth day of life in Case 2, in the absence of continued treatment with iodides, could reflect increased thyroxine production by the thyroids of these infants. It will be recalled that the serum PBI level is considerably elevated normally during the first week of life and slowly falls during the first year (28). The recorded values in Case 1 and Case 2 considerably exceeded the normal range at any age. In Case 3 the serum PBI level twenty-four hours after thyroidectomy was within the normal range. In this instance it seems most probable that all symptoms could be attributed to the respiratory obstruction alone.

Treatment. The treatment of congenital sporadic goiter of the newborn is in most instances symptomatic. Of primary concern is the maintenance of an adequate airway. The enlarged thyroid frequently compresses the trachea posteriorly. In the presence of dyspnea, stridor, retraction and cyanosis, surgical intervention may be necessary in order to prevent death from asphyxia. It is believed that subtotal thyroidectomy is preferable to tracheotomy, since the latter procedure may not adequately relieve the retropharyngeal obstruction. In the absence of severe respiratory difficulty, thyroidectomy is not indicated and the goiter will usually diminish in size within a few weeks.

If the infant appears to have thyrotoxicosis (tachycardia, diarrhea, failure to gain weight, exophthalmos and an elevated level of serum PBI), it may be necessary to employ parenteral fluid therapy, paregoric and iodides. Iodides should probably be used instead of the thiouracil drugs, particularly in infants born of mothers treated with the latter, for two reasons: 1. Iodides are more rapidly effective and less toxic and, since the goiter is usually transitory, they may be discontinued at an early date. 2. The goiter in the newborn infant may be due to excessive thyrotropin production secondary to placental transmission of thiouracil drugs; therefore it would appear wise to avoid further use of such compounds and thus reduce the possibility of continued thyrotropic overactivity.

The occurrence of hypothyroidism in the infant with neonatal goiter is uncommon; when present, appropriate substitution therapy is indicated. In many instances the infant is probably euthyroid and the goiter will subside spontaneously without therapy. Although the existence of "goitrous cretinism" cannot be ruled out, no case of this disorder has been described in which there was significant thyroid enlargement at birth.

In view of the widespread use of goitrogenic drugs (such as sulfa com-

pounds, thiouracil derivatives or ? cobalt) in the treatment of the pregnant woman, it might be anticipated that the incidence of sporadic neonatal goiter will increase. It is remarkable that under these circumstances the condition is not more common today. In order to avoid the development of neonatal goiter, it is advisable to review certain principles relative to the treatment of the thyrotoxic pregnant woman with thiouracil derivatives. 1. It is to be recalled that in normal pregnancy there is usually a "physiologic hyperthyroidism," with moderate elevation of the serum PBI level and the BMR. Therefore treatment should not be directed towards attaining levels of thyroid function characteristic of the nonpregnant euthyroid individual. According to Man (29), the concentration of serum PBI during pregnancy is approximately 60 per cent higher than in the normal nonpregnant adult. During pregnancy it may actually be advisable to correct thyrotoxicosis only partially with the thiouracil drugs, to a point consistent with the comfort and general well-being of the mother, and beyond this to permit slight deviations of the serum PBI level from the normal values *for pregnancy*. 2. If the hyperthyroidism in the mother has been reasonably well controlled, the thiouracil drugs should be withdrawn two or three weeks before delivery, provided this procedure is not attended by a significant clinical remission of the maternal disease. The substitution of iodides for the thiouracil derivatives may not be advantageous (Case 1), since iodides themselves may induce neonatal goiter when administered to the pregnant female. However, since the dosage of iodides required for the control of hyperthyroidism (1 minim of Lugol's solution daily) is considerably smaller than that generally employed, it may be that the lesser quantity would not affect the fetus. Ideal control of hyperthyroidism is often not achieved, however, with iodides alone.

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Letters to the Editor

IODINATED COMPOUNDS IN THE LYMPHATIC PATHWAYS LEADING FROM THE THYROID*

To the Editor:

In 1949, when Mr. Selwyn Taylor was working with us, we carried out some very preliminary work on I^{131} in the lymphatic pathways leading from the thyroid. This work is being repeated and, with the application of quantitative chromatography now in use for other studies in our labora-

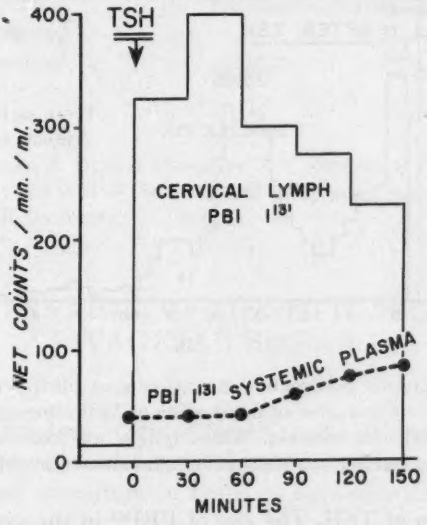

Fig. 1. The rise in the level of protein-bound radioactive iodine in lymph recovered from cervical lymphatics draining the thyroid area, before and after the administration of TSH.

tory, has been expanded. Further preliminary and perhaps important results have been obtained.

Large dogs were given I^{131}. One to four days later the animals were anesthetized and capillary cannulas inserted into the cervical lymphatics

* Aided by a grant from the American Cancer Society.

directly beneath the thyroid. Continuous collections of lymph were obtained. Lymph could thus be collected at a rate of up to 0.75 ml. per hour. In the initial experiments some years ago, it was found that the amount of I^{131} in the lymph increased markedly within thirty minutes after the intravenous injection of TSH. Serial samples of cervical lymph were collected for thirty-minute periods and protein-bound radioiodine (Smogyi precipitation) determinations were made on each. The results are shown in Figure 1. There was a marked rise in the PBI^{131} level in the cervical lymph after

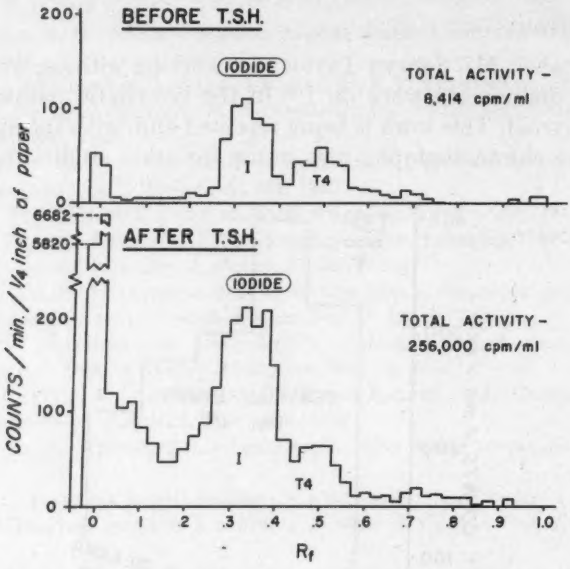

FIG. 2. Chromatographic pattern of iodinated compounds in lymph from the thyroid area. The samples before and after administration of TSH represent collections from the same continuously draining cannula. Whole lymph was chromatographed on paper strips in a descending fashion in a dioxane-butanol-ammonia system.

the administration of TSH. The rise of PBI^{131} in the systemic plasma was demonstrable somewhat later.

More recently, since we have had reliable methods of quantitative chromatography, the foregoing observations were repeated and carried considerably further; serial quantitative fractionations were made of lymph from many areas, as well as fractionations of venous and arterial plasma. It may be seen in Figure 2 that there is an enormous increase in the output of radioactivity in the lymphatic pathways from the thyroid after the administration of TSH, as previously demonstrated. This increase is predominantly due to a substance which does not move in a di-

oxane-butanol-ammonia system. Preliminary observations suggest that this may be thyroglobulin. The iodine (I) and thyroxine (T4) fractions are evident in the chromatograms. At about Rf .69–.75 there is a faint suggestion of triiodothyronine.

Simultaneous studies have been made on blood from the thyroid veins. Here we have found iodide and thyroxine, as Taurog *et al.* have described (1). TSH also causes an increase in the output of radioactivity in the blood from the thyroid veins. Because it currently seems impossible to measure the total volume of the lymphatic and the venous output from the thyroid, we are unable to determine the relative importance of these two routes of exit from the gland. In general it can be said that volume for volume the lymph (which is probably diluted by tributary pathways above the thyroid) contains more radioactivity than does the blood from the thyroid vein after the administration of TSH.

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DETERMINATION OF GENETIC SEX BY THE VAGINAL SMEAR

TO THE EDITOR:

Much interest has been devoted lately to the determination of genetic sex by studying the cell nuclei in skin biopsy specimens. This has proved a very useful clinical procedure in cases of hermaphrodism and "ovarian agenesis" (1–5).

During the routine examination of vaginal smears stained by the Papanicolaou technique, it was observed by us that in many instances the sex chromatin could be distinguished remarkably well in the nuclei of the vaginal epithelial cells, especially if these were clear and vesicular and showed a finely granular chromatin meshwork. As there are suitable nuclei in almost every smear, the sex chromatin body, characteristic of the female, could mostly be identified without difficulty as a conspicuous black dot usually lying at the nuclear rim. Only in rare instances, when all the cell nuclei were irregularly shrunken, necrotic or pycnotic, or when the super-

position of many cytoplasmic inclusions made identification difficult, could the chromatin corpuscles not be clearly demonstrated. Sometimes also an inadequate staining of the nuclei rendered interpretation impossible.

The former difficulty was typically encountered in buccal smears which, for a comparative study, had been taken from male and female volunteers. In the comparison of simultaneously obtained buccal and vaginal smears of patients, it became at once apparent that vaginal smears were generally much more suited for this kind of study. The same superiority in other respects has also long been recognized by cytologists. So far, we have no experience of the special staining technique for buccal smears that has recently been described by Moore and Barr (6).

CYTOLOGIC STUDY

A systematic investigation was then undertaken in a series of 49 cases of primary amenorrhea, unknown to the cytologist. In these cases, at least one vaginal smear (sometimes taken years before) was still available. From this series 4 cases with poorly prepared smears were discarded. Among the remaining 45, cytologic study revealed that there were 10 males and 35 females.

In 25 cases of the female group, the presence of ovarian tissue was proved by later spontaneous menstruation (6 cases), or upon investigation at laparotomy (19 cases). In 2 of the latter group, already listed as typical "ovarian agenesis" cases, the gonads were rudimentary. In 2 others (published elsewhere (7)), the typical morphology of this syndrome was lacking; in serial cuttings of a biopsy specimen from the gonadal rudiment, one showed some rare follicles, the other none at all. The 15 other patients had normal ovaries, but 6 of them had a rudimentary vagina.

The male group of 10 patients was even more interesting: 7 were listed as cases of "ovarian agenesis," and in 4 of these the presence of rudimentary gonads was proved at laparotomy. The 3 remaining males were a surprise: they all showed the typical "ovarian agenesis" morphology, but had not yet been listed under this diagnosis, because of lack of an increased urinary FSH titer. In 1 of them, the presence of "streak ovaries" had even been proved at laparotomy. The 2 others, as was found out afterwards, had been given estrogen treatment elsewhere, for one and five years, respectively.

These findings, resulting from the study of sex chromatin by the vaginal smear, clearly illustrate its diagnostic value, especially in primary amenorrhea. They also reflect the usefulness of this very simple and effective method. With a minimum of training one can soon learn to identify the nuclei in which the chromatin corpuscles may be visible. These corpuscles may even after a while be recognized when not lying at the nuclear rim.

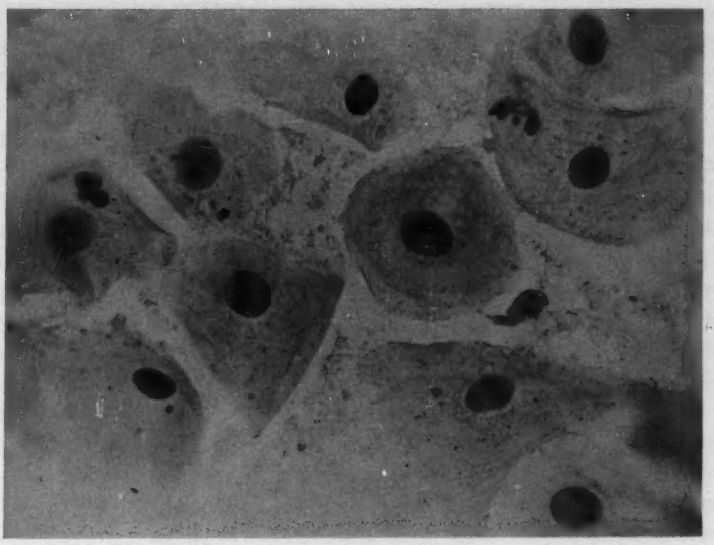

FIG. 1. Smear from a female case of "ovarian agenesis." Note sex chromatin bodies near rim of nuclei. (×800.)

FIG. 2. Smear from a male case of "ovarian agenesis." No sex chromatin bodies are visible. (×800.)

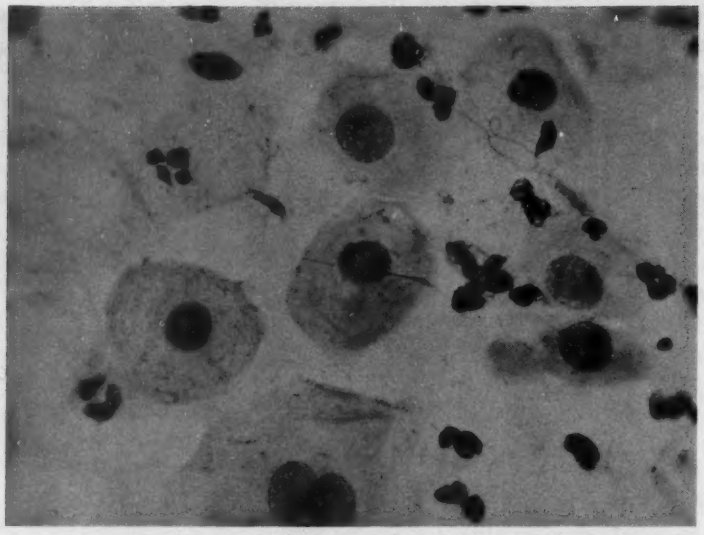

FIG. 3. Smear from a rudimentary vagina in a case of primary amenorrhea (female). (×800.)

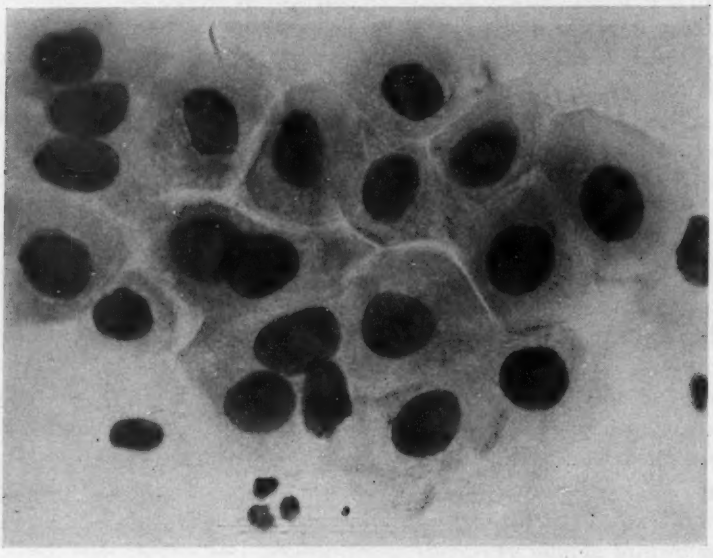

FIG. 4. Smear from the urogenital sinus of a female pseudohermaphrodite (adrenal hyperplasia). (×800.)

A much smaller black dot, already pointed out also in other studies as present in part of the male nuclei (8), can usually be differentiated without difficulty. The difference between sexes is in most instances so conspicuous, that counting and calculating become just as unnecessary as in every other qualitative or morphologic study.

Cases of "ovarian agenesis" lend themselves particularly well to this investigation, owing to a rather normal vagina and absence of estrogenic stimulation, resulting in the desquamation of basal cells with large, vesicular and finely granular nuclei (Figs. 1 and 2). However, even in a very shallow and rudimentary vagina, as in 6 of our cases, satisfactory smears can easily be obtained (Fig. 3). We had no difficulties in determining sex by smears taken from the urogenital sinus in 3 cases of female pseudohermaphrodism and from the extremely hypospadic urethra in 1 case of male pseudohermaphrodism (Fig. 4). Every cytologist should routinely check the nuclear sex chromatin, as this detail is an important acquisition in his field.

Thus, in the 12 patients in our series in whom the diagnosis was established as "ovarian agenesis" syndrome, there were 10 males and 2 females. This striking proportion is remarkably concordant with that in the group described by Grumbach *et al.* (9), who found 2 females in a total of 17 patients. The pooling of these two series results in 4 females in a total of 29 patients. It is therefore allowable, on a statistical basis ($p<.001$), to reject the hypothesis of an equal proportion of the sexes in such cases. This does not necessarily prove that male embryos are more sensitive to the intra-uterine noxes that result in "ovarian agenesis" syndromes in later life. One might as well postulate that genetic females less often survive to manifest lesions of this kind; or, on the contrary, that nature less often succeeds in their complete intra-uterine castration.

Undoubtedly, the valuable landmark furnished by the discoverers of the "sex chromatin," will help greatly in the research that should broaden our comprehension of different sexual phenotypic aberrations.

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THYROTOXICOSIS IN A MONGOL

To the Editor:

Recently we have had the opportunity to examine and study a mongol in whom the typical clinical and laboratory features of thyrotoxicosis had developed. Search of the literature indicates that such a coincidence is unique. We present details of our findings in this patient (Case 1, Table 1) and summarize the results of investigations in 4 additional mongols in whom, by clinical assessment, thyroid function was normal (Cases 2 to 5, Table 1).

CASE REPORT

J. H., female, aged 23 years, was admitted to the hospital on August 11, 1954. She had been in a mental institution since the age of 16 years. No abnormalities had been noted at that time other than the stigmata of mongolism and slight exophthalmos. At 21 years of age she had become mentally and physically more sluggish. Desiccated thyroid, 1 grain daily, had produced no improvement over several months and it was discontinued.

In the year prior to admission she had lost 14 pounds in weight. Exophthalmos had become more marked, and she had had several "bilious" attacks. Sexual excitability had increased, accompanied by frequent masturbation. Menstruation was normal.

On admission the typical features of mongolism were noted, namely, slanting palpe-

bral fissures, persistent plica marginalis fetalis, flat forehead and nose, small mouth and enlarged tongue, high palate, deficient circum-umbilical abdominal musculature, and the "four-finger line" in the hands. Mental deficiency was gross. In addition there was marked bilateral exophthalmos, and a marginal ulcer was present on the left cornea, with conjunctivitis. The optic fundi showed degenerative myopic changes with circumpapillary atrophy and choroido-retinal degeneration. The limbs were hypotonic and there was a fine tremor of the outstretched hands. The skin was warm and moist. A small smooth goiter was palpable. The pulse rate fluctuated between 62 and 92 per

TABLE 1. SUMMARY OF 5 CASES OF MONGOLISM

	Case 1	Case 2	Case 3	Case 4	Case 5
Sex	F	F	F	F	F
Age in years	23	29	8	14	22
Clinical assessment of thyroid status	Hyperthyroid	Euthyroid	Euthyroid	Euthyroid	Euthyroid
Additional clinical features	Congen. heart dis.	Congen. heart dis.	Congen. heart dis.	Congen. heart dis.; diabetes mellitus	Dwarfism; chronic diarrhea
Tests of thyroid function:					
B.M.R. (% above standard)	82	14	—	—	—
Plasma cholesterol (mg./100 ml.)	87	—	114	136	—
Serum PBI^{127} (μg./100 ml.)	9.2	—	—	—	—
Radioactive iodine (I^{131})					
24-hour thyroidal uptake					
% of dose	88	35	37	58	57
48-hour urinary excretion					
% of dose	10.5	45.8	47.2	36	—
48-hour plasma PBI^{131}					
% of dose/liter	0.78	0.09	0.32	0.24	Negligible
Tests of pituitary function:					
Urinary 17-ketosteroids (mg./day)	4.7	9.5	2.3	6.0	—
Urinary acid-hydrolysable formaldehydogenic substances (mg./day)	3.1	—	1.4	1.2	—
Urinary follicle-stimulating hormone (mouse units/day)	<10	<20	<20	—	—

minute, and the blood pressure varied from 100/70 to 140/70. A systolic murmur, maximal down the left sternal border, was heard at all the heart valve areas. The breasts were normally developed, but the pubic and axillary hair, although present, was rather scanty. Hemoglobin was 14.5 Gm. per 100 ml.; erythrocyte sedimentation rate (Westergren), 7 mm. in 1 hour; plasma cholesterol level, 87 mg. per 100 ml.; and serum protein-bound iodine level, 9.2 μg. per 100 ml. Basal metabolic rate tracings were made with difficulty, and a reading of 82 per cent above standard was obtained.

Radioactive iodine (I^{131}) studies showed a gland uptake at twenty-four hours equivalent to 88 per cent of the dose. At forty-eight hours the total plasma I^{131} was 0.86 per cent of the dose per liter, of which 0.78 per cent per liter was protein-bound. Only 10.5 per cent of the dose was excreted in the urine in forty-eight hours.

The daily urinary excretion of 17-ketosteroids was 4.7 mg.; acid hydrolyzable formaldehydogenic substances, 3.1 mg.; and follicle-stimulating hormone, less than 10 mouse units.

Radiography of the skull showed mongoloid configuration with congenitally enlarged emissary foramina posterior to the mastoid processes, and failure of fusion of the posterior area of the first cervical vertebra. No abnormality was seen in the orbits or in the chest.

A diagnosis of thyrotoxicosis was made and treatment with Lugol's iodine (5 minims three times daily) was instituted. At the time of her discharge in September 1954, thirty-

one days later, there was marked clinical improvement in her condition; she was much less excitable and her weight had increased by 11 pounds. She was next seen in December 1954. In the intervening three months, due to a misunderstanding, she had had no therapy, and her weight had fallen 12 pounds. Clinical signs of thyrotoxicosis were apparent and the exophthalmos had become more marked. Iodine medication was recommenced, and by April 1955 she had regained 9 pounds in weight. Her general state once again was more settled, and the exophthalmos had receded slightly.

COMMENT

On clinical assessment, 4 of our patients (Cases 2 to 5) were euthyroid, and the radioactive iodine tests supported this opinion. In 1 patient (Case 1) the clinical diagnosis of thyrotoxicosis was made and was supported by the elevated B.M.R., high level of serum protein-bound I^{127}, low level of blood cholesterol, and abnormal results with radioactive iodine. The clinical improvement on Lugol's iodine and the subsequent relapse when the iodine was omitted provided additional confirmatory evidence.

Kearns and Hutson (1) have previously reported normal thyroid function, as assessed by radioactive iodine tests, in infant mongols; they compared the results found in a series of mongols with those in normal newborn infants, and contrasted them with those obtained in cretins in whom thyroid function was impaired. The results, indicative of normal thyroid activity for their age in 4 of our mongols, are in agreement with the experience of these authors.

In discussions on the etiology of mongolism, genetic, endocrine and metabolic defects are usually postulated, but the etiology remains obscure. Penrose (2) suggests that a concatenation of maternal and fetal genotypes, and of certain precipitating environmental influences, which may be nutritional or hormonal, may cause fetal growth to be disturbed in a special way. Particular attention has been paid to the possibility that an endocrine disorder may be the cause of the condition. For example, Benda (3) considers that mongolism is due to pituitary hypofunction, and that consequent failure of thyrotropic hormone may result in failure of thyroid function.

It is obvious that our experience provides no support for an opinion that mongolism takes its place among the thyroid deficiencies. Further, none of our 5 patients showed any clinical evidence of hypopituitarism; laboratory tests supported this opinion in the 4 patients so investigated. We may, therefore, reasonably conclude that, as far as our studies go, there is no evidence for an endocrine basis, pituitary or thyroid, for mongolism.

SUMMARY

1. Details of clinical and laboratory findings are presented in the case of a mongol in whom thyrotoxicosis developed. Such a coincidence is unique.

2. Findings in 4 additional mongols who were euthyroid are summarized.

3. None of the patients showed evidence of pituitary deficiency.

4. No evidence for an endocrine basis for mongolism, namely pituitary or thyroid deficiency, was found.

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ISOLATION OF $\Delta^{1,4}$-PREGNADIENE-3-20-DIONE-11β-17α-21-TRIOL FROM THE URINE OF PATIENTS TREATED WITH METACORTANDRACIN

To the Editor:

The urinary excretion of "reducing corticoids" in 4 patients receiving metacortandracin[1] ($\Delta^{1,4}$-pregnadiene-3-11-20-trione-17-21-diol) in a dosage of 30 mg. daily, was studied by paper chromatography.

The 24-hour urine specimens were collected with thymol as a preservative, extracted with chloroform prior and after incubation with β-glucuronidase (Sigma Chemicals), washed with 0.1 N NaOH and water and chromatographed on Whatman No. 54 paper in the toluene-propylene glycol system, according to the method of Burton, Zaffaroni and Keutmann (1).

In this system, metacortandracin appeared to have a mobility (R_E) of 0.70 ± 0.02 relative to that of cortisone, as compared to hydrocortisone (0.37) and tetrahydrocortisone (0.21) (2).

The chromatograms of all 4 patients receiving metacortandracin disclosed a spot with R_E 0.23, which absorbed ultraviolet light at 254 mμ,

[1] We are indebted to Roussel (Paris) for a generous gift of metacortandracin, and to the Pfizer Co. Belgium for a generous gift of pure $\Delta^{1,4}$ pregnadiene-3-20-dione-11β-17α-21-triol.

showed no fluorescence after treatment with 2N NaOH, and reduced blue tetrazolium and TPTZ.

This was evidence for a steroid possessing both an α-ketol side-chain and a Δ^4-3-ketogroup with a preserved double bond at C_1, because reduction of this bond would have produced cortisone. Cortisone shows fluorescence after treatment with 2N NaOH, and was not observed at this place on the chromatogram. Thus the unknown steroid appeared to be most likely the Δ^1 homologue of hydrocortisone, *i.e.*, $\Delta^{1,4}$-pregnadiene-3-20-dione-11β-17α-21-triol.

A pure sample[1] of $\Delta^{1,4}$-pregnadiene-3-20-dione-11β-17α-21-triol showed exactly the same R_E as the unknown substance. This was true also after acetylation. Furthermore, a mixed chromatogram disclosed only one spot. Both the pure sample and the unknown had the same ultraviolet spectrum in sulfuric acid. This is strong evidence that the unknown metabolite of metacortandracin was $\Delta^{1,4}$-pregnadiene-3-20-dione-11β-17α-21-triol.

This conversion of metacortandracin is analogous to the conversion of cortisone to hydrocortisone and indicates that the enzymatic system which reduces the 11-oxy group is not influenced by the double bond in C_1. The analogy goes further in that, just as with cortisone, this reduction does not result in a loss of activity. The reaction appeared to be reversible.

On the same chromatograms we found two spots which reduced blue tetrazolium. However, they had no blue fluorescence in ultraviolet light. Research is in progress for further identification, but as yet we have good reason to believe that these are the Δ^1 analogues of tetrahydrocortisone and tetrahydrohydrocortisone. This would mean that the metabolism of metacortandracin follows, at least in its early phase, the same metabolic pathway as cortisone.

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The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 7-8-9, 1956.*

The Committee on Local Arrangements is Dr. Norris J. Heckel as Chairman with Drs. M. Edward Davis, Sam Taylor, J. P. Greenhill, and Harley E. Cluxton as members of the Committee.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

The Council requests that members send suggestions for nominations for the elective offices of the Society to the Secretary. Those desiring to do so should send this information to the Secretary immediately for transmission to the Nominating Committee.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Dwight J. Ingle, 950 East 59th Street, Chicago 37, Illinois, not later than *February 1, 1956. It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that these will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-

authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter perfect, since there will be no opportunity for proofreading by authors.

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years

(1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1956 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, May 3, 4 and 5, 1956.

SECOND WORLD CONGRESS ON STERILITY AND FERTILITY

The Second World Congress on Sterility and Fertility of the International Fertility Association will be held in Naples, Italy, May 18–25, 1956. About 180 scientific papers covering all phases of infertility are scheduled to be presented. All interested physicians are invited to attend.

For all information regarding travel, registration, and hotel accommodations, write to Maxwell Roland, M.D., Chairman, Liaison Committee, 1141 Eastern Parkway, Brooklyn 13, N. Y.

THE AMERICAN ORTHOPSYCHIATRIC ASSOCIATION, Inc.

ANNOUNCEMENT OF ANNUAL MEETING

The American Orthopsychiatric Association will hold its 33rd Annual Meeting at the Hotels Commodore and Roosevelt in New York City, on *March 15, 16 and 17, 1956.*

Orthopsychiatric theory and practice in five broad fields will be presented in approximately 65 papers by representatives of the wide range of professional disciplines and settings practicing in the field of orthopsychiatry. These broad fields include:

1. Schools and Mental Health.
2. In-patient and Out-patient Psychiatric Treatment of Children.
3. Adolescence and Juvenile Delinquency.
4. Psychiatric Clinic Management.
5. Adult Psychotherapy.

The opening Presidential session will have as its keynote the Challenge to Orthopsychiatry; perspectives for the profession, for the community, and scientific and moral perspectives.

Twenty workshop sessions with limited attendance provided for by advance registration will offer opportunity for active participation of those attending. Selected mental health films will be shown for two days of the meeting. Technical and commercial exhibits related to the field will be on display.

The American Orthopsychiatric Association, founded in 1924, is a membership organization of psychiatrists, psychologists, psychiatric social workers and members of allied fields, including education, anthropology and sociology. Members come from all parts of the United States, Canada and abroad.

Inquiries about the program, reservations, exhibits and other matters should be directed to Dr. Marion F. Langer, American Orthopsychiatric Association, 1790 Broadway, New York 19, N. Y.

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH ANNOUNCES PUBLIC HEALTH SCHOLARSHIPS

Scholarships for the Academic Year 1956–57 will be granted to individuals of high professional promise in awards ranging from part tuition to tuition plus a stipend, according to the qualifications and financial needs of the applicants. The Scholarship Funds are limited and are primarily intended for citizens of the United States. In general, preference will be given to applicants under 35 years of age.

Scholarships are available to those in the following categories who wish to obtain post-graduate education in the field of public health or in one of the basic sciences related to public health: Physicians, Dentists and Veterinarians; Industrial Physicians; Public Health Nurses with a college degree and satisfactory field experience; Social Workers with a master's degree from an approved school of social work and acceptable experience in the field of medical or psychiatric social work; Health Educators with a college degree, training either in health education or in the natural and social sciences and experience in general education or community health work; Dietitians with a college degree and satisfactory field experience; College Graduates who have concentrated in one of the Natural Sciences or in Engineering (environmental aspects), for example, Biologists, Biochemists, Nutritionists, Microbiologists, Parasitologists or Biostatisticians.

Scholarship applicants must be eligible for admission to the School as a candidate for one of the following degrees: MASTER OF PUBLIC HEALTH, DOCTOR OF PUBLIC HEALTH, MASTER OF SCIENCE IN HYGIENE, DOCTOR OF SCIENCE IN HYGIENE, MASTER OF INDUSTRIAL HEALTH.

A Catalogue of the School, Admission and Scholarship applications, and further information may be obtained by writing the Secretary, Harvard School of Public Health, 55 Shattuck Street, Boston 15, Massachusetts.

Scholarship applicants must return completed admission and scholarship applications to the Harvard School of Public Health by *March 1, 1956*. Scholarship awards will be announced May 1, 1956. Under exceptional circumstances awards will be made at other times.